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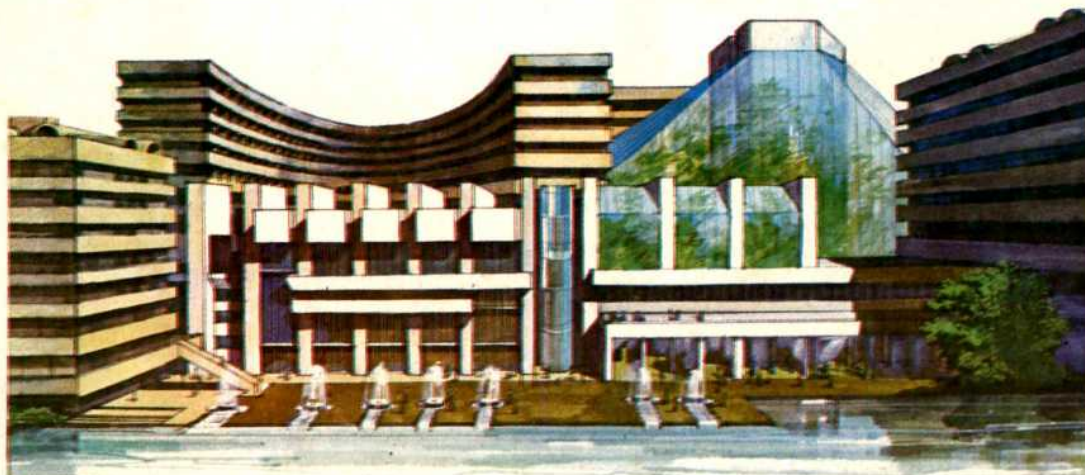
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No easy route to non-proliferation

NOWHERE has the interrelationship between politics and technology become more entangled than in discussing the relationship between the spread of nuclear energy and the proliferation of nuclear weapons. To candidates in the 1976 US presidential campaign, the issue seemed relatively straightforward: the greater the availability of plutonium — either through the reprocessing of spent nuclear fuel or through the deployment of fast breeder reactors — the greater the chance that some of this would be diverted to military purposes. Domestically, this philosophy became relatively easy to institutionalise in the Nonproliferation Act of 1977. Internationally it has fallen on sceptical ears.

The final report of the International Nuclear Fuel Cycle Evaluation (INFCE), initiated by President Carter over two years ago in an attempt to generate an international consensus behind the US strategy, has ended up a long way from endorsing his position. Admittedly there are parts of the final report from which the US can take satisfaction. For example, the nations taking part in the study agreed in general that sensitive nuclear facilities capable of producing weapons-grade material, such as reprocessing and enrichment plants, should be limited to as few countries as possible. And the report also confirms the US view that reprocessing is not a prerequisite for the disposal of nuclear wastes, and of marginal economic significance for thermal reactors.

US negotiators are also claiming solace from the fact that, although they have been unable to secure agreement on slowing down the deployment of fast breeders in the interests of non-proliferation, they have at least placed the issue more prominently than before on the international agenda. But this victory is, in many ways, a Pyrrhic one. INFCE did not agree to support any generalisations about the comparative proliferation risks of different fuel cycles. Indeed during its deliberations, the international deployment of fast breeder programmes, particularly in countries such as France concerned about guaranteed future access to uranium supplies, seems to have increased.

What the debate has lacked is a climate of trust. And this is as true for the relationship between the US and its fellow nuclear suppliers as between the suppliers and their customers. Many countries were upset, not so much by the US strategy, but by the apparent strong arm tactics by which it was seeking to impose it unilaterally. President Carter declared his intention that the US "Should provide strong leadership, using our own exports of nuclear fuel and technology to persuade other countries not to seek or sell the technology to make bombs." Many took exception to this attempt to use economic hegemony to force the rules of the game covering international trade in nuclear technology — particularly at a time when the US and European industries

seemed about to compete for lucrative third world markets.

The value of INFCE is that it has placed many of the issues raised by President Carter in broader perspective. We now know, for example, that there is unlikely to be any one technical fix to the proliferation question, and that the thorium-cycle does not necessarily offer any great advantages in this respect. We also know that the lack or adequacy of uranium supplies cannot be demonstrated simply. But whether we are any closer to reducing the dangers of proliferation is unclear.

One problem is that, whatever the detailed conclusions and recommendations of the report, any message that fast breeders are safe could, unless carefully handled, lead to unwarranted complacency. The report has successfully argued that political issues with regard to restricting proliferation cannot be reduced to technical questions relating solely to the availability of fuels or reactor technology. But it conveys an optimism about the ability of political institutions to cope with the problems that may, in practice, turn out to be equally overstated.

Perhaps this optimism is no greater than that with which President Carter swept into office three and half years ago, determined to demonstrate that controlling the hazards of nuclear power — or for that matter eliminating infringements of human rights — was little more than a question of political will. But such arguments were conceived in the calmer days of the mid-1970s, when the short-term stability of international relations made it possible to argue in terms of long-term strategies.

In the aftermath of Iran and Afghanistan, the climate has changed dramatically. Yet the irony is that, just at a time when the US is trying to restrain countries such as Pakistan and India from a rush to nuclear weapons, and arguing the need for effective international controls, it is engaged with other western nations in boosting its own nuclear arsenal.

Given this situation, it is difficult to see emerging the "Bargain of confidence" urged last month by a study group convened by the Royal Institute for International Affairs, with support from the Rockefeller Foundation. Indeed it is the reality of self-interest that needs to be addressed if we are to make genuine progress towards reducing the threat of nuclear war. Just as there are no technical fixes to the proliferation problem, so we should not be fooled into thinking there are any quick political fixes.

Doing what we can to raise the global standard of living, and reducing the tensions that flow from inequities in the distribution of wealth and power, will in the long run be the only way of removing the incentive for nuclear war. In the short term, efforts to conclude a comprehensive ban on the testing of new weapons, and to maintain pressure for arms limitation treaties such as SALT 2, would be a welcome show of good faith. A world built on conflicts and tensions — with or without fast breeders — can only encourage the instabilities that point to nuclear disaster. □

United Kingdom

Chemical company suppresses dioxin report

WORKERS exposed to 2,3,7,8-tetrachlorodibenzodioxin (dioxin), between 1968 and 1971, during manufacture of the herbicide 2,4,5-T, at the Derbyshire site of the UK company Coalite and Chemical Products Ltd, could face an increased risk of developing cardiovascular complaints, according to confidential documents in *Nature's* possession.

The documents — reports of detailed clinical and laboratory investigations on a number of dioxin-exposed workers at Coalite — suggest that the company has been falsely reassuring about the health of this particular sector of its workforce. According to a spokesperson for the Health and Safety Executive (HSE), the Executive also believes that the Coalite workforce has no long-term health problems. However, its view is based only on reassurances given by the company and not on a study of the medical reports which Coalite has not published and which the HSE is powerless, in law, to demand from the company because it no longer manufactures the product, 2,4,5-T.

Ironically, it was pressure from the HSE which forced Coalite to carry out the investigations in the first place shortly after the Séveso accident in 1976. Included in the study were 126 individuals, 41 of whom were known to have been exposed to dioxin, and showed symptoms of chloracne; 54 of whom might have been exposed to the chemical; and a supposed control group of 31. One of the studies compared the blood chemistry of the different groups. The author of the report of that study is Dr Jenny Martin, a lecturer in Occupational Medicine at the University of Manchester and a consultant chemical pathologist at Chesterfield Royal Hospital when the study was commissioned.

The results of that study show that the dioxin-exposed group has a greater incidence of impaired liver function as measured by the enzyme gamma-glutamyl transpeptidase. Furthermore when the results for serum cholesterol, triglyceride, high density lipoprotein etc were subjected to multivariate analysis — under the guidance of the Department of Probability and Statistics at the University of Sheffield — they showed a significant difference between the dioxin-exposed group and the controls. In the dioxin-exposed group, levels of serum cholesterol and triglyceride were higher and high density lipoprotein lower than in the controls. These are factors commonly held to imply an increased risk of cardiovascular disease.

None of this information is to be published, however. According to Martin, Coalite decided not to publish it on the

advice of Dr Kenneth Crow, a consultant dermatologist at St Margaret's Hospital, Swindon. She claims that Crow had told her that he had advised Coalite not to publish on the grounds that he was not happy with the statistics used in the report.

Crow, one of the world's leading authorities on chloracne, was involved in the treatment of such cases at Coalite. When approached by *Nature* to comment on the study, he said that he did not know which study was being referred to and that he would have to see it before he could comment. However, he strongly denies ever having advised Coalite not to publish the results of any study. *Nature* has approached Coalite for its side of the story, but with no success. Coalite stopped talking to journalists shortly after the Séveso accident in 1976.

Some related information, however, has been published. Instead of using an age and occupation-matched control group, Coalite chose to bolster the number of controls by including management staff undergoing a regular lipid screen at the time. When the study was commissioned, Martin was unaware of the composition of the three study groups. When she later learnt that the control groups had not been properly matched, she arranged to re-examine eight of the Coalite workers suffering from chloracne and to compare their blood chemistry with a matched control group.

The results of this second investigation, published in a letter to the *Lancet* (24 February, 1979, page 446), also show increased serum cholesterol and reduced serum high density lipoprotein in the dioxin-exposed group. The differences were considerably more marked than in the original, larger study. However, they were not statistically significant, a point which Martin notes, but says is simply due to the small numbers of subjects involved.

Perhaps the most extraordinary aspect of the story, however, concerns a burglary at Martin's house. Shortly after publishing her letter in the *Lancet*, Martin's house was broken into and the detailed medical records of the eight Coalite subjects were removed from her filing cabinet. Martin reported the theft to the local police constabulary but as she had no idea why anyone would want to steal this information the police investigation never got off the ground. A police spokesperson at Macclesfield confirms that the theft had been reported, that the case was not closed, but that as there was no clues about the motives for the theft, it was unlikely that it would ever be solved.

Martin told *Nature* that she had been

very distressed when she discovered the theft of the material for the second survey. She has no duplicate copy so the work is now lost.

She was surprised that *Nature* had obtained a copy of her original Coalite report, and having confirmed its authenticity said she was most unhappy that Coalite were not publishing the data. She said the company's decision was a major reason for her carrying out a second study and reporting it to the *Lancet*.

Coalite did, however, release an abbreviated form of the original report to one of the unions involved with the workforce at its Bolsover complex — the Association of Scientific Technical and Managerial Staffs. The abbreviated report is totally different from the original. In addition to its selective reporting, the union version says there were no statistically significant differences between the dioxin-exposed group and the controls, a statement which is quite untrue.

The most worrying aspect of this affair however, is the position of the HSE. The Executive has said that it is satisfied that the Coalite workers have not been unduly affected by their exposure to dioxin. Yet, if it has never seen the results of the clinical investigations, how can it express such a view? It says that it has to rely on the good faith of the company on this matter. According to an HSE spokesperson, when a product is no longer manufactured the Executive has no legal powers to demand medical records of workers. It is abundantly clear that if the Executive is to do its job properly it should have access to this information and should be given the legal powers to demand it. **Alastair Hay**

Unions want 2,4,5-T ban

THE UK Trades Union Congress has called for an immediate ban on the use of 2,4,5-T, pending a thorough investigation into its effects by the Health and Safety Executive. The National Union of Agricultural and Allied Workers has already advised members not to handle it. 2,4,5-T is widely used by the Forestry Commission in the UK, which has rejected such alternatives as manual or mechanical clearing as too costly. Two County Councils in England have also banned the pesticide.

The Ministry of Agriculture's Pesticides Advisory Committee has investigated 2,4,5-T eight times, and stuck to its conclusion that it is safe as long as handled in accordance with instructions. The TUC has condemned these enquiries as inadequate. □

United States

Patent law changes opposed in the Senate

ATTEMPTS to liberalise US patent laws by granting universities — and small businesses — exclusive rights to patents from federally-funded research have run into stiff opposition in the Senate on the grounds that they are selling out the American tax-payer.

Many leading research universities have campaigned actively in support of a bill, introduced by Senator Birch Bayh and Senator Robert Dole, which would grant such exclusive rights to any university or small business able to show that it is prepared to spend additional funds on encouraging the exploitation of the patent.

However, when the bill, which has been unanimously approved by the Senate Judiciary Committee, was introduced on the floor of the Senate last month, it ran into unexpectedly vehement opposition from Senator Russell Long, powerful chairman of the Finance Committee and long an opponent of apparent monopoly practices.

Senator Long threatened to filibuster the bill, hinting that this might place in jeopardy the progress of windfall profits legislation on the oil industry. The bill was quickly withdrawn from the debate by Senate leaders, worried that it might otherwise be lost, but is expected to be brought up again shortly.

Meanwhile Senator Long sent a letter to his Senate colleagues last week outlining his opposition to measures which he said represented a "radical and far-reaching giveaway" by the federal government, and would if passed "wipe out every law on the books which reserves for the public the paid results of [federally-financed] research".

Patent law revision has become one of the cornerstones of current attempts both within Congress and by the Carter administration to create an environment considered more conducive to technological innovation by private industry.

The Bayh Bill attracted over 50 co-sponsors when it reached the floor of the Senate. In speaking to the bill, Senator Bayh repeated a common complaint that although the government lacked the resources to develop and market products arising from federal research, it was unwilling to relinquish patent rights in a way that would encourage others to do so.

Similar arguments were heard from others in the debate. Senator Strom Thurmond pointed out that universities had successfully licensed 33% of the patents that they held, whereas 95% of the patents owned by the government had never been exploited. And Senator Edward Kennedy argued that, by helping raise the general level of economic competition, the legislation "would help America retain its

competitive edge in technology and innovation".

To refute charges that the government is selling out investments in research made by the US tax-payer, the bill contains a formula by which funds will be returned to the government from those inventions that eventually enjoy substantial success.

But this has not dampened Senator Long's opposition. In his letter to Senate colleagues he agrees that economic growth and increased productivity "require the most rapid dissemination of scientific and technological knowledge" — but argues

that allowing firms to file private patents would do the opposite by "bottling up" technical information.

In support he quotes Nobel Prize winner Wassily Leontief, Admiral Hyman Rickover, and Michael Pertschuk, Chairman of the Federal Trade Commission, who told a Senate committee in 1977 that there was no basis for claims that giving away title to private contractors promotes commercialisation of government-financed invention, and that the available evidence "shows the opposite". □

Third World research institute loses Congressional approval

THE Carter administration seems to have failed in its attempts to establish a new body to promote scientific and technological research efforts relevant to the needs of developing countries, known as the Institute for Scientific and Technological Cooperation (ISTC).

Delegates from the Senate and the House of Representatives, meeting last week to resolve differences of opinion over foreign aid spending in 1980, agreed to allocate \$12 million to the Agency for International Development (AID) to support programmes of scientific and technical cooperation — but rejected the proposal to establish a new institute to carry out such programmes.

Thus although the institute legally came into existence last October, following agreement between the two legislative bodies on authorising legislation, all of its activities will now be incorporated within AID, with responsibilities that were to have been delegated by Congress to the director of the ISTC now being assumed by the administrator of AID.

The rejection of the ISTC by Congress, which seems inevitable even though the conference report could theoretically be objected to by either House, is a major blow to the administration, particularly since the institute figured prominently in the US presentation to the United Nations Conference on Science and Technology for development in Vienna last August.

From the beginning, administration officials have argued that the new institute, plans for which were first announced by President Carter in a speech in Caracas in 1978, would not be acceptable to Congress if it was run by a totally independent board — an alternative proposal actively canvassed by some scientists — but that a new body operating under the broad umbrella of AID should be acceptable.

Many in Congress have not been

convinced by the arguments for a new institute. In particular, Senator Peter Deconcini last summer persuaded the Senate to reject authorisation plans for the institute on the grounds that it would create unnecessary additional bureaucracy.

This decision was reversed in meetings with the House of Representatives on the authorising legislation. But Senator Deconcini used the same argument last October to persuade the Senate to cut off funds for the institute, and this time his arguments prevailed in conference, although House delegates managed to argue the extra money for the AID budget.

Administration officials are debating whether, now ISTC has been authorised but not funded, they should push ahead for the \$95 million requested for the institute in the 1981 request. But such a move could jeopardise the \$15 million requested for the new Science and Technology Fund being set up by the United Nations Development Programme, which has already been cut back to \$10 million by a House subcommittee.

Those who have been following the changing fortunes of the ISTC in Washington last week expressed disappointment, but little surprise, at the outcome, since the debate over the institute has become bogged down in a political climate increasingly unsympathetic to providing assistance to Third World countries.

However some now feel that the time may be ripe for other initiatives. The National Science Foundation, for example, has recently announced a new programme to encourage US universities to establish links with Third World research groups. Others are suggesting that private foundations might be persuaded to support a scaled-down version of the original ISTC proposal.

David Dickson

International exchange

Hamburg forum a success — of a kind

"It was a success. All twenty speakers at the closing session agreed that it was a success. The fact that everyone stayed and there is a final statement is a sign of success".

Such was the summing up of the executive secretary, Dr Klaus Gottstein to *Nature*, when, after two weeks work and a marathon all-night session that ran on past the official closing date, the Hamburg Scientific Forum managed to hammer out a final statement that was more than diplomatic lip-service to peace and détente.

For in addition to noting that since the signing of the Helsinki Final Act there had been a "significant expansion of scientific cooperation . . . greater in some areas than in others", observing that "different levels of scientific development . . . should be taken into account when planning scientific cooperation", and laying the foundations for convening a further forum in the future, the final statement contained three proposals on subjects which had been the focus of controversy throughout: human rights, the international training of young scientists, and the freedom of scientific exchange and communications.

While there was no reference to Sakharov in the final statement — although the US delegate did raise the issue again in his closing address — the human rights issue did find a place there in general terms.

Increased scientific cooperation, said the statement, can only be achieved "by respect for all the principles and by full implementation of the relevant provisions of the Final Act. All participating states are therefore urged to observe the spirit and the letter of the Final Act, particularly with respect to the conditions essential for scientific cooperation."

"It is furthermore considered necessary to state that respect for human rights and fundamental freedoms by all states represents one of the foundations for a significant improvement of these mutual relations and of international cooperation at all levels."

One subject which the Yugoslav delegate, Dr Drago Ocepek, had earlier associated with human rights was the international training of young scientists. Several delegates had endorsed this theme, including Dr Helge Gyllenberg of Finland and Dr J J Went of the Netherlands. This approach, however, is not popular with the Soviet Union, which tends to view visits abroad as a reward for achievement rather than a stimulus to further effort. Indeed, throughout the two weeks, the Soviet delegates had shown a marked reluctance to discuss any practical details of the logistics of exchange, claiming that the agenda should be confined to professional

discussions on the selected subjects — energy, urbanisation, food production and cancer, cardiovascular and virus diseases.

Nevertheless, international "training courses for young scientists . . . that would enable them to study new science and methods for shorter or longer periods" found a place in the final statement, with the recommendation that "information about these facilities . . . should be disseminated as widely as possible".

The other major barrier to international exchange — restrictions on the free circulation of scientists and information had also produced vigorous confrontations. There were the routine complaints about Soviet scientists who fail to arrive at conferences where they are scheduled to speak — countered,

somewhat irrelevantly, by an accusation from the Soviet Union that in 1976 the University of Geneva had refused to let an Iranian "assistant" in the law department deliver lectures in Marxism. Full freedom of exchange, said the Soviet and East German delegates, was only possible in conditions of complete disarmament.

From the Danish delegation, Dr Maaloe had described the work of the ICSU Committee on the Safeguard of the Pursuit of Science, and stressed the scientific and economic losses caused by unnecessary secrecy. All this was condensed in the final statement to a clause urging "equitable opportunities for scientific research and for wider communications and travel necessary for professional purposes".

Vera Rich

US academy suspends exchanges with Soviet Union

THE council of the National Academy of Sciences has voted to suspend for a period of six months all bilateral symposia, seminars, workshops and new initiatives with the Soviet Academy of Sciences, in protest at the internal exile from Moscow of physicist Dr Andrei Sakharov.

The first meeting to be affected by the academy's decision will be a conference on the interactions between laser beams and matter which was to have taken place at the University of Arizona in early March, and to which 20 Americans and 15 Soviet scientists had been invited.

Three other meetings over the next six months will also be affected, including meetings between scientists from both sides

on basic research, on physics and on experimental psychology. However the council has stressed that its decision is not meant to affect individual contact between US and Soviet scientists, which it says "are matters properly left to the consciences of the participating individuals".

The academy's decision was agreed by a vote of 10 to 3, although some of those who voted against are thought to have pushed for firmer measures, such as extending the suspension of exchanges to one year.

In a telegram sent after the council's meeting to Academician A P Aleksandrov, President of the Soviet Academy of Sciences, Dr Saunders MacLane, Vice-President and acting chairman of the NAS council expresses "our profound hope that the safety and freedom of movement of academician Andrei D Sakharov and his family will be protected".

Dr MacLane also says that the policy adopted by the council — which has been organising exchanges with the Soviet Union for 21 years — reflects the strongly held views of many US scientists. "Our council hopes that the circumstances that have led to the adoption of that policy will soon change so as to permit restoration of the full exchange programme which we have, until recently, viewed with great satisfaction".

The council adds that it sees "no long term national benefit in modifying scientific exchanges to every political action and reaction", but that it is taking action following repeated requests from its members. Dr Sakharov was made a foreign associate of the academy in 1973.

David Dickson



United Kingdom

London medical colleges for the chop

THE Westminster Medical School, and the pre-clinical courses at King's College and the Royal Free Hospital should close, according to a London University working party whose report was published last week. In addition the British Postgraduate Medical Federation would be disbanded and five of its smaller institutes would lose their separate identities. Chaired by Lord Flowers, the committee was charged with rationalising expenditure on medical education in the university while retaining student numbers and staff.

Savings of £6 million a year would result, says the report: £1 million on maintenance of the premises to be vacated; £1 million on administration; £1 million on academic services; and a further £3 million "from the eventual rationalisation of academic departments and academic posts". The present cost of the schools to the university is some £51 million a year. The savings would be redistributed among the remaining medical schools.

In terms of research, the principal casualty of Flower's proposal would probably be King's College — one of only two schools in the university (the other in University College) to operate its pre-clinical teaching on a multi-faculty university campus rather than in a hospital.

"Research is not threatened directly" says Peter Baker, professor of physiology at King's, who estimates that King's receives 24% of all the Medical Research Council and Science Research Council grants for pre-clinical science subjects to the University of London Medical Schools. But if King's loses its medical students, staff members are likely to be reduced and their research with it.



Lord Flowers: finding a way to rationalise expenditure.

Baker feels that the working party has been unduly influenced by a desire to bring pre-clinical teaching within hospitals, in direct opposition to the recommendations of the Royal Commission on Medical Education (the Todd report of 1965) — which stressed the need for a broader-based, multi-faculty organisation for medical teaching.

The battle lines are drawn, Baker believes, between the medical profession which would like to see students exposed to hospital life as early as possible, and the scientists who believe that a medical student requires a thorough grounding in scientific method. Also at stake, says Baker, may be the money involved in pre-clinical education — twice the amount spent on the clinical phase.

When clinical and pre-clinical education are combined 'vertically' within a teaching hospital, says Baker, with the division of funds controlled by hospital committees, "clinicians are very effective in giving the pre-clinicians little money". Pre-clinicians were not represented on the Flowers committee, Baker notes.

The report will be considered by the university for a decision in July. □

High Energy Physics

LEP by 1986?

THE Large Electron-Positron collider, LEP, could produce its first beam by 1986 — three years ahead of the original target, the European Organisation for Subnuclear Physics, CERN, is expected to announce later this week. A Committee of Council meeting last Friday approved this advanced programme, although CERN member states have not yet received a formal proposal.

If CERN members are favourable, there could be a decision within a year to build LEP — while keeping to a 'constant' budget. Pressure from the Italian delegation, plus the prospect of competition from the United States have led to CERN's new sense of urgency.

The attitude of Professor Herwig Schopper, who is likely to be elected CERN's next director-general in June, may also have contributed. Professor Schopper told *Nature* recently that LEP could be built within five years of a positive decision, "but we will have to cut out all the frills". The minimum 'one-sixth' LEP defined in earlier CERN designs could be trimmed, thinks Schopper, to a 'budget-LEP' without changing the size of the tunnel or compromising the chase for the intermediate vector boson. "Building electron accelerators is very different from building proton accelerators" he says. "In our last accelerator at DESY [PETRA] most of our calculations were proved wrong. It will be the same with LEP."

Building LEP will thus be an experiment. Sophisticated orbit correction mechanisms should be built when needed rather than designed *a priori*. Financial savings could also be found in the experimental halls. **Robert Walgate**

ARMS proposes security for 60% of untenured researchers

THE UK Association of Researchers in Medical Science has prepared a document showing that 60% of present contract workers could be placed in tenured career tracks with no additional expenditure and no reduction in the workforce. The plan calls for the creation of a pool of tenured researchers to be funded out of the £81.5 million now used to support contract work by five main sources; the MRC (£24.8 million), the Department of Health and Social Services (£18.3 million), charities and trusts (£22 million), pharmaceutical companies (£11.9 million) and the Royal Society (£4.5 million). ARMS proposes the creation of research institutes based at

universities or medical schools typically employing 85 researchers, 50 of whom would be on tenured tracks and 35 post-doc. The cost per institution would be £1.29 million per year based on an average salary of £9,000 plus 25% for pension and insurance plus £2,500 running expenses plus a 10% institutional surcharge.

Surveys of three industrial laboratories show that total running costs of a research establishment of 2000 employees averages £20 million a year, a figure that compares favourably with ARMS' estimates. The total cost of the ARMS scheme nationally for the estimated 4500 researchers currently employed on short term contracts

would be £68 million. The proposed 10% institutional surcharge would be used to build up an operating fund for tenured researchers out of the estimated £26 million currently supplied by charities and trusts who cannot commit their money on a long term basis.

Representatives from ARMS discussed the proposal last week with Professor James Gowans, Secretary of the Medical Research Council who requested that it be taken to the universities first for their reactions. Gowans told *Nature* that "ARMS is writing to the wrong address. If they get a deal from the universities then I'll have a look at it". **Joe Schwartz**

NEWS IN BRIEF

Spanish science lurches ahead

SPANISH scientists have raced feverishly to meet a 1 March deadline for applications for newly released research funds. Announced in mid-January by the Comisión Asesora de Investigación Científica y Técnica, the amount of 1600 million pesetas (£10.6 million) is the first money made available to researchers since 1977. In addition the commission announced the formation of a committee of 20 to map out future Spanish research strategy. Researchers fear that the committee may impose a straightjacket on the fragile state of basic research out of an inability to realistically assess what kind of research is in "the national interest". A three year plan for restructuring research backed by 50 billion pesetas (£350m) will be tabled in the parliament this Spring.

Canadian research threatened

THE Science Council of Canada has warned that threats to the quality of Canadian higher education, where university enrolment is currently decreasing even though the number of 18-24 year olds is increasing, could have a severe impact on the future strength of Canadian research.

The report* says that for the past ten years, the financial resources for teaching and research provided by both the provinces and the federal government have generally been less than sufficient to keep up with inflation, leading to the serious erosion of research facilities built up in the 1960s.

It says that in addition to seeking more money, universities will have to make a number of hard decisions. These include the elimination of courses and programmes where the number of students and the resources for teaching or research are inadequate for productive scholarship of the highest quality; the transfer of faculty and facilities to other universities; the consolidation of graduate work in specific disciplines into joint centres embracing more than one university; and the common use of major facilities.

**University Research in Jeopardy: The Threat of Declining Enrolment. Available from the Canadian Government Publishing Centre, Supply and Services Canada, Hull, Quebec.*

Pharmaceutical company to rent space at Yale

MILES Laboratories Inc, a subsidiary of the West German chemical and pharmaceutical company Bayer AG, is to rent space in a building allocated to Yale University's department of biology to

establish an institute for pre-clinical pharmacology.

Initially seven or eight scientists and technicians are expected to work on the research and development of new pharmaceuticals, primarily in the cardiovascular field, in a section of Yale's Osborn Memorial Laboratories, which the company has agreed to renovate as part of its payment to the university.

"This agreement is especially significant for the basic biological sciences, since close ties with industry have not existed previously at Yale," Dr Frank R. Ruddle, professor of biology and human genetics, and chairman of the biology department, said in announcing the agreement. "Because of new technical and theoretical developments in biology, we believe that such relationships will be mutually beneficial and therefore more common in the future."

Stanford announces new centre for electronics

THE University of Stanford has announced plans for a new Center for Integrated Systems, bringing together in a single research laboratory individuals with experience in physics, materials science, electrical engineering and computer research to work on very large scale integrated systems (VLSI) for industry and business management.

"Our goal is to make Stanford the premier centre in the world for VLSI research" said Dr James F Gibbons, professor of electrical engineering, in announcing plans for the \$15 million centre.

The three goals of the new centre are: to produce approximately 100 MSc and 300 PhD graduates per year ("tomorrow's technological leaders" according to Professor James Meindl); to promote research, including the development of working design automation capability and computer-managed fabrication facilities; and to offer, via instructional television and videotapes, new courses, conference and workshops "designed to keep scientists and engineers now employed in industry abreast of rapid developments in the field." Funding will come principally from government and industry sources.

Fusion beam injection success

SCIENTISTS at the University of California's Lawrence Berkeley Laboratory last week reported that they have successfully tested a neutral beam injection system which will be used to heat the plasma of the Tokamak Fusion Test Reactor at Princeton Plasma Physics Laboratory, due to start operating in December 1981.

According to Dr Kenow H. Lou, project manager for the injection system at LBL, deuterium beams were successfully accelerated to 120,000 electron-volts, producing seven million watts of power in pulses lasting 0.5 seconds. The team is now studying ways of increasing the duration of the beam to 1.5 seconds.

The Princeton Tokamak will use four such neutral beam injection systems, the prototype of which has been built as a collaborative effort between LBL and the Lawrence Livermore Laboratory, which carried out the beam line engineering. Following the successful tests — described as a "major milestone" in the US fusion energy programme — the prototype system will remain in operation to develop further information and experience which may affect TFTR operations.

UK short of basic grade nuclear inspectors

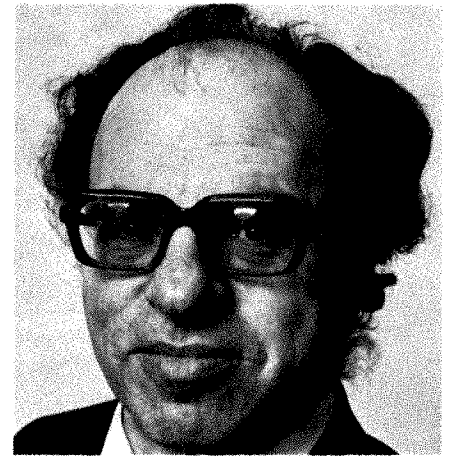
THE Nuclear Installations Inspectorate is 53% below its full complement of basic grade inspectors according to information released last week by the Department of Employment. In answer to a parliamentary question by Frank Hooley (Labour, Sheffield) the department revealed that the NII was having difficulty recruiting structural, electrical, mechanical and instrumentation engineers and has a shortfall of 14 out of its complement of 26 positions. A Health and Safety Executive official told *Nature* that routine work was not affected but that in the review of the pressurised water reactor specific areas of work might have to be subcontracted to the Safety and Reliability Board of the UK Atomic Energy Authority. The 61 principal inspectors and 26 inspectors of the NII are responsible for surveillance of 11 nuclear power stations, three radio-chemical centres, five nuclear fuel production works and the UKAEA's facilities at Windscale, Dounreay, Springfields, Winfrith, and Harwell.

CERN opens data transmission experiment

THE European STELLA experiment to transmit scientific data by satellite between sub-nuclear physics laboratories was initiated today in Geneva. Directors-General of the European centre for nuclear research, CERN, the Internal Market and Industrial Affairs Commission of the European Communities and the European Space Agency took part in a ceremony inaugurating fast and reliable data transmission between CERN and laboratories in Hamburg, Saclay, Oxford, Pisa, Dublin and Graz. The STELLA system will transmit data at 1 megabit per second using the European telecommunications satellite OTS-2.

No technical fix to a political problem

THE International Nuclear Fuel Cycle Evaluation ended on 29 February with a plenary session that adopted the reports of all the study groups. **Hermann Bondi** (right) Chief Scientist at the UK Department of Energy and leader of the British delegation to INFCE, assesses its work



THE INFCE reports naturally reflect many points of view, yet they were unanimously agreed. Some countries had suggested 'technical fixes'; some claimed that energy independence was necessary, and that the whole argument about non-proliferation was just a device to keep a vital new technology firmly confined to the most advanced states. But it was agreed that there is no technical fix to the political problem of proliferation. There is no fuel cycle that presents no such risks and dangers. On the other hand the working groups looked closely at the sensitive areas; how they could be made less risky, what could be done to improve their security, and what institutional measures could be evolved which would further increase international re-assurance.

I concentrate on four areas: the availability of nuclear fuel, the economics of the fuel cycles, the risks of proliferation, and the terms and conditions of nuclear supply agreements. Safety and environmental questions were not addressed in any great detail.

INFCE assumes a fairly high rate of growth in nuclear generating capacity. Compared with about 125 GW of capacity today, INFCE projects 850 to 1200 GW in

the non-Communist world in 2000, and 1800 to 3900 GW in 2025. With use of the once-through cycle and on its high projection of growth, INFCE concludes that the lifetime uranium requirements of the reactors installed and in operation by 2000 will approach current estimates of known uranium resources (5 mTU). These figures have been criticised, notably in the US, for being unduly pessimistic about uranium availability.

But the figures are not everything. INFCE rightly stressed that the uncertainties in these forecasts are great — not just in the prediction of nuclear demand and of the extent and accessibility of geological reserves of uranium. The uncertainties in the political availability of uranium are greater perhaps than for any other commodity. The message is simple. If countries are to be confident that there will be enough uranium to fuel their reactors over the next 30 to 40 years, acceptable solutions to political and environmental problems limiting the exploitation of uranium must be found, new sources of uranium must be discovered and brought into production, and reactor strategies that conserve uranium must be adopted by at least some of the big users.

The scope for new discoveries of uranium is good. Estimates range from 6.6 to 14.8 mTU. If these resources can be discovered, brought into production, and the uranium made available internationally, supply should meet the requirements of INFCE's perhaps more realistic low projection of nuclear growth to beyond 2025. But the extent to which this will happen is uncertain. The more pessimistic a country, and the more dependent it is on uranium imports, the more likely it is to want to move to the early introduction of reactors that conserve uranium, such as advanced thermal reactors and fast reactors.

Fast reactors offer the prospect of nuclear development eventually almost free from uranium supply constraints. So while INFCE does not say that the early introduction of fast reactors is essential on resource grounds, it does illustrate the impact of decisions about reactor strategy — and their timing — on uranium supply and demand. Such decisions, like many in energy policy, depend largely on a country's perception of its need to be

insured against uncertainties in fuel supplies.

In discussions on the economic aspects of the fuel cycles it became clear that considerations vary from country to country. No fuel cycle can be said to have a universal economic advantage over the next few decades. Recycle into light water reactors is of only minor economic advantage, but may offer the benefits of reducing reliance on imported uranium. On the other hand, if the capital and fuel cycle costs of fast reactors can be brought down, fast reactor recycle could offer considerable advantages.

INFCE came to no definite conclusion on when the fast reactor would compete economically with thermal reactors. The uncertainties in the future price of uranium and of fast reactors, and regional variations in uranium supply, mean that countries will take different views about when to proceed from thermal to fast reactors.

Much of INFCE's discussion centred on two related subjects: how the risk and the fear of weapons proliferation can be reduced, and how to make nuclear energy widely and assuredly available.

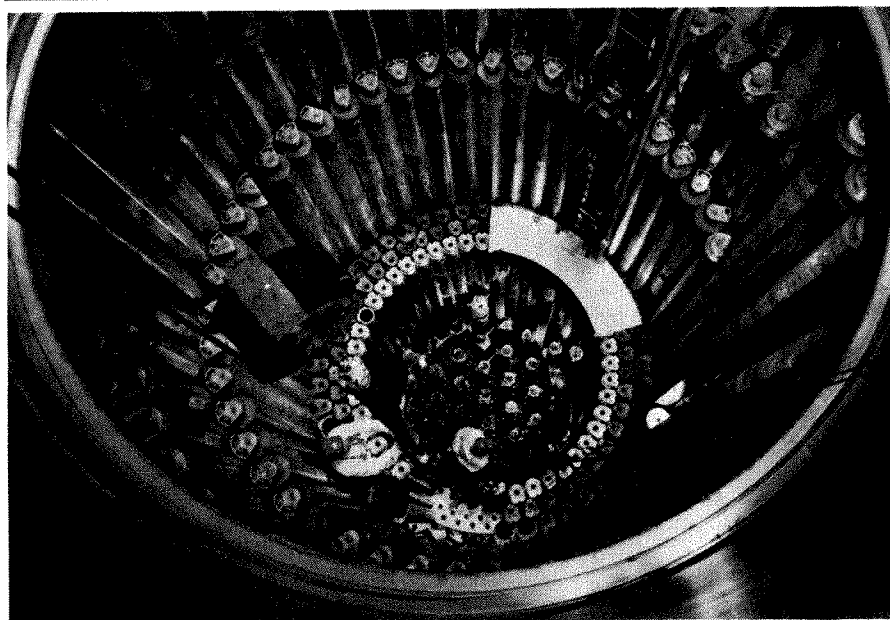
From the start it was recognised that a decision to construct nuclear weapons is political, and that the misuse of the civil fuel cycle is not the easiest or cheapest route to acquiring them. INFCE was a technical, not a political evaluation. The reports, therefore, look only at the way in which a civil nuclear programme might help a country make a nuclear explosive.

The misuse of fuel cycles that involve the handling of large quantities of weapons-usable material — not just plutonium, but highly enriched uranium (HEU) or uranium 233 — might offer a path. But discussions in the Working Groups and in the TCC showed that comparisons between the proliferation risks of the various fuel cycles were extremely complex. How, for example, does one assess the relative proliferation

Background and participants

INFCE was set up in Washington in October 1977, in response to heated disagreements between states over the nuclear fuel and technology trade. In particular, President Carter had aroused anxiety among developing nations in April that year when he called a halt to the separation and use of plutonium, implying the deferral of reprocessing and confinement to a once-through cycle.

Sixty-six countries took part in the discussions; industrialised and developing; weapons and non-weapons states; East and West; nuclear suppliers and customers; those that had signed the Non-Proliferation Treaty and those that had not. Five international organisations also took part. The Evaluation was not, however, a negotiation. The Organising Conference set up eight working groups, linked by a Technical Co-ordinating Committee.



Refuelling the UK's Prototype Fast Reactor at Dounreay, Scotland. Facilitating supply of this fuel (plutonium oxide) without risking weapons proliferation was one of INFCE's major concerns

risks of the various fuel cycles were extremely complex. How, for example, does one assess the relative proliferation risk of an enrichment plant, which produces HEU for a research reactor, with that presented by spent fuel (which necessarily contains plutonium) stored in ponds where it becomes increasingly accessible as its radioactivity decreases?

INFCE concludes that the proliferation risks of fuel cycles cannot be compared in the abstract, but depend on the specific arrangements. No single judgement about the relative risks of proliferation can be made which will be universally and permanently valid. The reports look at those fuel cycle activities that are sensitive and concentrate on measures to reduce the risks of proliferation arising from them.

INFCE examines the various technical options for reducing the proliferation risks of the back end of the fuel cycle. Measures to reduce the presence of weapons-usable material in separated form in the fuel cycle, such as the co-conversion of the uranium and plutonium solutions produced at reprocessing plants directly into mixed oxide; measures to use radioactivity to protect these materials from diversion, for instance, by spiking fuel with high energy gamma emitters; and measures to protect them by the use of physical barriers, such as providing extra containment around reprocessing plants.

Measures using radioactivity as a protective barrier not only involve considerable environmental and economic penalties, but also make safeguards more difficult because of the material accountancy problems created by the intense radiation. The other measures, though perhaps desirable for future plants, turn out by themselves to have only a limited influence on proliferation risks.

INFCE thus could not identify any

potential technical fixes in this field, but stressed that research reactors could and should be converted to require only uranium of low enrichment. This was the only useful technical measure emerging from a full discussion of the proliferation risks arising so seriously in enrichment.

More promising are institutional measures. Multinational fuel cycle ventures can offer a country seeking fuel cycle services a more economic way of meeting their needs, and are a potentially useful measure for reducing proliferation risks. INFCE points that a first step towards such institutional arrangements would be for countries with large fuel cycle facilities to offer services to others. This would help postpone the need for the latter to build their own fuel cycle facilities.

But perhaps the most promising institutional measure is a scheme for international plutonium storage so that plutonium was only stored under international supervision and released solely for safeguarded peaceful uses. This could have important non-proliferation advantages and INFCE applauds the work being done in the International Atomic Energy Agency to establish such a scheme.

The central feature of the non-proliferation regime, however, is the network of agreements, generally implemented by the IAEA by which states accept the international system of inspection or "safeguards" on their nuclear activities. INFCE sees no problems with the techniques applied to existing operating plants, but foresees an important need for development work on the techniques for safeguarding plants handling large quantities of weapons-usable material. A crucial point is the need for security objectives to be considered at the design stage of any plant.

One thing, however, that INFCE does

not go into is how to win wider acceptance of safeguards, mainly because this is a political question not in INFCE's remit. But there is an area of INFCE's work which may provide part of the answer. Working Group 3 was devoted to the question of how to improve "assurances of supply" — assurances that once a nuclear supply contract has been signed, neither government will interfere in its performance. In recent years some suppliers, notably the US and Canada, because of growing worries about proliferation, have cut off previously agreed supplies. This has reduced the credibility of such arrangements, and has tended to make customers more determined to make themselves self-sufficient in nuclear supplies and services in the long run.

Working Group 3 has looked at the way in which suppliers can be given confidence that their non-proliferation concerns can be met, while at the same time giving customers the confidence that their supplies will not be interrupted. This is the central theme of non-proliferation policy, and the strength of feeling among the developing and other customer countries about their need for nuclear energy makes its solution of crucial importance. Working Group 3 suggests that a consensus on the non-proliferation conditions of supply could first be expressed in "common approaches" to bilateral agreements, which might later evolve into a more formal multilateral agreement on the terms and conditions of nuclear trade. These ideas should be followed up, and the IAEA offers an ideal forum for doing so.

The findings of INFCE do not reflect only the views of a few large industrialised nuclear countries. The Evaluation has respected the views of the countries with small nuclear programmes, the customer and developing countries.

Looking at the resource arguments, it describes the reasons why countries wish to keep open the option of plutonium recycle. Looking at the economic arguments, it shows why many countries look ahead to fast reactors so as to extract the maximum energy value from limited uranium resources. Looking at non-proliferation questions, it shows how pointing accusing fingers at different fuel cycles or seeking alternatives can uselessly divert attention from the more fruitful task of improving safeguards and creating suitable institutions. And finally, it suggests how work towards an improved consensus on the conditions of nuclear trade would both promote the aims of non-proliferation and help ensure that nuclear energy is available to meet the world's energy requirements.

It is a triumph of common sense and of common purpose that the very wide cross section of countries represented in INFCE, created by the US initiative, could arrive at this consensus, which while in no way underwriting the original American specific measures, points a way forward. □

Europe embraces the fast breeder reactor

The International Nuclear Fuel Cycle Evaluation has given a boost to the fast breeder reactor. But while the US is rejecting the breeder, others are embracing it, writes **Robert Walgate**

FRANCE'S fast breeder programme is already many years ahead of any other nation's. Last week Electricité de France announced an order for a further two fast reactors of 1500 MW each to supplement Super-Phenix, a commercial demonstration reactor due to produce electricity in 1983, so confirming the French lead.

But while France may be ahead in practice, other European countries are with her in spirit. Last month the Council of Foreign ministers of the nine-nation European Community drafted a resolution urging member states which have fast breeder programmes to "get on with them", and offering "appropriate" EEC support. Already, Euratom loans are available to countries developing fast breeders.

Moreover, the Assembly of the Council of Europe, a loose association of 18 European states, recently adopted a resolution that "current programmes in Europe for the development of fast breeder technology to the point of commercial-sized demonstration plants should be continued".

Dr Walter Marshall, Deputy Chairman of the United Kingdom Atomic Energy Authority, and a prominent member of INFCE, argued last week that countries with an advanced nuclear programme, such as those of Europe, should concentrate on fast breeders, leaving

thermal reactors to other countries.

Dr Marshall, who once described INFCE as "an exercise in proliferation" because it would help communicate sensitive technologies from the most to the least nuclear of its 66 participants, said that such a regime "would not guarantee the non-proliferation of nuclear weapons . . . but it does give the maximum opportunity to governments . . . to secure the most proliferation-resistant regime possible."

Last week the UK House of Lords debated the fast breeder issue, prompted by the Council of Europe resolution. Viscount Thurso, whose fief includes the Dounreay prototype fast reactor, compared progress.

"Our own PFR deliberately contains fuel elements of commercial size such as will be used in a commercial demonstration fast reactor, and employs boiler and coolant pump designs of a commercial sort which will only have to be scaled up slightly for CDFR. Super-Phenix will have to use fuel elements and boilers of designs hitherto untested in practice, and will in consequence have a fairly large question mark hanging over it."

In the US "EBRII is still running but FFTF is doing criticality tests only and work on CFBR has stopped . . . The state of progress in Russia indicates that BN600 is not yet critical and may not be operational until 1983 and although they are preparing to start on BN1600, they have a long way to go with its design, especially in the field of fuel technology."

In Japan "where they very much need atomic energy . . . they are very far behind schedule. Monju, scheduled for operation in 1986, is not yet in building and it is only the equivalent of our PFR."

"In Germany, the KNK11, is only a converted thermal reactor and SNR300 is delayed by legislation; SNR2 is only a gleam in their eye. In France alone is progress being maintained, although in my view they are behind us in fuel element and boiler design and have an unproved fuel cycle."

Britain should thus move rapidly to the promised public enquiry on the CDFR, argued the Viscount, and in this he was supported by most speakers. Only two strong voices were raised against — those of Lord Avebury, an engineer and a Liberal, and Lord Bowden, an ex-Secretary of State for Education and Science.

Lord Avebury welcomed the debate not because of the immediate need for the fast breeder but "as a warning of the powerful onslaught which is to be expected from the

nuclear lobby if, and when, the government initiate the public inquiry".

"I think that the adoption of breeder technology, plus the reprocessing of the spent fuel from thermal reactors, which of course involves the separation of plutonium, would mean that by the middle of the next century . . . there would be 25 or 30 nations with at least a primitive weapons capability."

And Lord Bowden questioned the economics of the reactors. "No-one can be found to deny that a fast breeder reactor will cost very much more than a conventional type. The ratio has been variously estimated at five times. The Germans have found that their own estimated cost has escalated ten times since they first started on the work."

The price of uranium must rise dramatically, said Lord Avebury, if the fast reactor was to be economic — perhaps from its present level of \$40 per pound to \$218 per pound, according to a study prepared by the US Arms Control and Disarmament Agency. But at these prices, argued Lord Bowden, there would be an increase in the size of economic uranium by a substantial factor, perhaps sufficient to equal the maximum breeding factor attainable in a fast reactor.

This breeding factor (the energy obtainable per pound of uranium in a fast breeder as compared with a thermal reactor cycle) "will be less than a dozen times in practice on any system which has so far been proposed and worked out in detail" and not the theoretical factor of 70 commonly quoted. "The Americans are very shrewd in their assessment of economic potential" said Lord Bowden "and they have decided there is no case for developing the fast breeder for several years to come . . . So I am afraid that if we go ahead with the fast breeder in Europe we shall repeat almost precisely the story of Concorde: it will be a technical triumph and an economic catastrophe."

Lord Bowden urged the government instead to consider the Canadian CANDU reactors "which get three of four times as much power per tonne of uranium" as pressurised water or advanced gas-cooled reactors as a way of conserving uranium. "The amount of power we shall in practice get from fast breeders is nowhere near the theoretical amount postulated. It will almost certainly take at least 20 years before the fast breeder can double the amount of fissile material it has to start with. It will take several tonnes of plutonium to start off a fast breeder and, when it has been started, the plutonium and the blanket have to be taken out and reprocessed chemically many, many times."

"I beg the government" said Lord Bowden "to consider this as an alternative". □

● **Maurice Bazin reports from Rio de Janeiro on the Brazilian response to INFCE.**

The response is one of relief and diplomatic excitement. The controversial multi-billion dollar nuclear agreement with West Germany, signed in 1975, which envisages the construction of up to eight plants of 1,200 MW by 1990, accompanied by uranium enrichment and fuel reprocessing facilities, remains unaffected.

"There was a consensus at the meeting that its conclusions will have no moratorium effect on our nuclear programme and will not serve as a pretext for modifying existing treaties" declared Hervaldo de Carvalho, director of the National Commission for Nuclear Energy (CNEN) who attended the INFCE sessions. "The results will be distributed among all countries without discrimination, but no country will be forced to take special measures as a result of the technical exercises carried out by INFCE."

CORRESPONDENCE

Dioxin, an emotive word

SIR, — The tragic murder of the production director of the trichlorophenol plant in Séveso demonstrates most dramatically that “dioxin” has joined a select group of words including “cancer” “nuclear” and “fluoride” which inspire fear and trigger emotions that require the explanations of psychologists not a chemist.

The ease with which traditional standards of rigour in proof can be abandoned when discussing toxicity should be a warning to all serious scientists. The article by David Dickson (31 January, page 418) can do nothing but reinforce the almost mediaeval atmosphere of mystic suspicion which seems to have enveloped this subject.

Pentachlorophenol is probably not one of the most widespread pesticidal chemicals in domestic use in the United Kingdom, other chlorophenols used in disinfectants could perhaps claim that honour, but it is quite widely used as a wood preservative and particularly in the United States. It has been used for more than forty years, and long before the present emphasis on worker safety, so that a very large number of workers have been exposed to considerably greater dosages than any relevant to the “cases” quoted in the article. There is no suggestion that the handling of pentachlorophenol-based wood preservatives, nor even pentachlorophenol itself, has been a major health hazard to operatives in the industry.

Surely if a material utilised at extremely low concentrations in contact with a consumer were to give rise to such dramatic hazards, then we could look for a large number of fatalities or significant debility in workers in the industry. This is simply not the case.

It is important to stress that the most toxic dioxin — tetrachlorodibenz-p-dioxin, has never been found in industrial pentachlorophenol (of modern manufacture) and the accuracy of analysis is now at a confidence level of one part in ten million.

Octachlorodibenz-p-dioxin, which is a known contaminant, is less toxic than pentachlorophenol itself. To claim that little is known of the physical and chemical toxicological properties of other dioxins is unfair, and perhaps the interested reader should refer to “*Pentachlorophenol Chemistry, Pharmacology and Environmental Toxicology*”, editor K. Tanga Rao, “*Chlordioxins — Origins and Fate*” by Ethyl H. Blair, and “*Dioxin — Toxicological and Chemical Aspects*”, edited by Flaminio Cattabeni, Aldo Cavalario, Giovanni Gallis. The hexachlorodioxins, which are found in technical pentachlorophenol of the quality now available in Europe, are at a level of about five parts per million, and the most toxic isomers are at proportionately lower levels. Their chronic toxicity is well established and it would be necessary to take more than the lethal dose of technical pentachlorophenol regularly in order to run the risk of future development of cancer. In effect the acute toxicity of pentachlorophenol protects against long term hazard.

The approach of certain environmentalists and American attorneys seems to centre on contaminants, even if present in extremely small quantities overlooking the accepted acute toxicity of the main ingredients. Furthermore they seem to believe that pentachlorophenol is applied in isolation and disregard co-solvents, solvents, carriers, resins, pigments and dyes, all of which form part of preservative formulations, the majority of which will certainly not have been

investigated for impurities nor their toxicology determined with anything approaching the thoroughness applied to pentachlorophenol. Nor is any mention made of the potential hazards to man of the organisms which pentachlorophenol controls.

Your readers can be assured that there is no iceberg, the divers have been down these many years and would have found it. Can I plead that we remember that all chemicals are toxic, it is only a matter of determining the dose?

Finally, I revert to my opening remark, can any psychologist offer an explanation of this fear and the profound emotions it releases?

Yours faithfully,

J. DAVID

Catomance Ltd, Welwyn Garden City,
Hertfordshire, UK.

World energy needs of the future

SIR, — R.B. Temple, in a letter (7 February, page 520) virtually as long as my (necessarily limited) review of *World Energy — Looking Ahead to 2020* (15 November, page 344), says many things I would readily endorse.

It is for the reader to judge whether I was sufficiently critical of the energy strategy proposed by the Conservation Commission of the World Energy Conference. My emphasising, through direct quotation as a form of shorthand, of the Commission's conclusion that their assessment required “doubling nuclear power plant capacity every six years”, was indeed meant to signal the reader to the far-fetched numbers this implied and which Dr Temple has calculated. No disagreement here, whatsoever, especially as the latest official UK estimates of new reactor capital costs are nearer £1000/kW, or £1 × 10⁹ for a 1000 MWe plant.

As for my own position in the energy debate, I would suggest an examination of Chapter 5 of *World Futures — The Great Debate* (ed C. Freeman and M. Jahoda), Martin Robertson, Oxford, 1978 which was reviewed by Lord Ashby in *Nature*, (November 9, 1978, page 144). I suspect Dr Temple and I would share much common ground.

Yours faithfully,

J. H. CHESHIRE

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Sussex, UK

Nuclear energy and the world economy

SIR, — R.B. Temple, who wrote a letter in your 7 February issue (page 520) commenting on the problem of feeding world economy with nuclear energy has been trapped in a famous pitfall, which is to assume that the size of a certain machine, in the particular case that of a nuclear reactor, is God given and time invariant.

Now, if we analyse energy systems or transportation, or chemical ones, we find that there exists a very tight link between the size of a plant and that of the market, in most cases the link being that of simple proportionality. So, if one increases electricity consumption by say a factor of 1000, which happened for example in the US from the beginning of the century to present, the number of power

stations stays constant (or more precisely decreased somewhat) because the stations' size grew with the market. There are presently about 3000 electric power stations in the world, of a certain size and connected to grids, and presumably a smaller number will be present in the year 2020. Consequently the number of plants to be constructed every year if we assume a life of 30 years will be 3000:30 or 100 per year at regime, which is not an extraordinary figure if we think it spread over the world. If we choose an energy vector different from electricity to carry around the energy, the number of reactors will depend on the cost of transportation. If we take hydrogen as an example, transportation costs in the proper context are an order of magnitude lower, and this makes generation possible in fewer and larger machines — say 100 for the whole world, with a replacement ratio of 3 per year. Does this still sound too many?

I am not entering into the complex question of financing energy systems development. I am just attracting attention to the fact that final consumers now pay for their energy something in the range of \$1.5 × 10¹²/year.

Yours faithfully,

C. MARCHETTI

International Institute for Applied Systems
Analysis, Laxenburg, Austria

Fast reactor safety and the fire at Beloyarsk

SIR, — Vera Rich's piece (31 January, page 420) based on a clandestine report of a fire at Beloyarsk in the USSR associates the fire with the fast reactor (BN600), at that site, and implies faults in the safety arrangements for that and, by implication, other fast reactors.

Beloyarsk is the site of two graphite moderated steam-cooled reactors, which were completed some years ago, and of the fast reactor BN600 which, at the time of the alleged fire, was still at least 12 months short of completion. The assumption that the fire, if any, might have led to the escape of radioactive sodium from the primary circuit of this reactor is, therefore, questionable, to say the least.

BN600 is a pool type reactor in which the core, pumps and primary heat exchangers are submerged in sodium contained in a strong double-skinned reactor tank. There is, therefore, no circuit, in the physical sense, which could “rupture” and “cause an explosion scattering radioactive material in a cloud . . .”. Vera Rich's reference to “the 1957 Kyshtym disaster” is equally inappropriate in the light of our ignorance of the nature, cause, or extent, of this incident.

It is not clear what implication the reader is meant to draw from the remark that “the fire at the Shevchenko fast breeder . . .” was not technically a “nuclear disaster”. This topic has previously been ventilated in your columns and information has been supplied which demonstrates both that this fire was no sort of disaster and that no radioactivity was involved since the circuit which leaked was a secondary sodium circuit external to the reactor.

Fast reactor safety is of public importance and, in your columns at least, deserves a more objective treatment than is displayed in Vera Rich's article.

Yours faithfully,

R.D. SMITH

Fast Reactor Development Directorate, United
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UK

NEWS AND VIEWS

The emergence of modern man

from Gail Kennedy

THE emergence of our own species remains one of the most elusive problems in palaeo-anthropology. Our increasing knowledge about very early hominids, such as the australopithecines, only serves to emphasise our lack of understanding of our own more immediate origins. In recent years, however, a few specimens have been found which give us tantalising views of the emergence of *Homo sapiens* from *Homo erectus*, the presumably ancestral species. One such discovery is reported in this issue (page 55). This well-preserved skull, Laetolil Hominid 18, was recovered in 1976 from the Ngaloba Beds at Laetoli, Tanzania. These beds are in the same area and stratigraphically overlie the Laetolil Beds in which Mary Leakey has found the fossil remains and footprints of early australopithecines.

L.H. 18, dating to about 120,000 years ago, shows a combination of advanced and primitive features. The overall expansion of the skull seen in the vertical parietals, rounded occiput and cranial capacity (1200 cm³) places it nearer modern sapients than to earlier *H. erectus*. In contrast to these more modern features, the large brow ridges, low, sloping frontal, marked bone thickness and certain features of the cranial base place L.H. 18 nearer to the ancestral group. Similar combinations of primitive and advanced characters are seen in other 'archaic' sapients from eastern and southern Africa such as the Omo skulls from the Kibish Beds in Ethiopia and the Broken Hill (Kabwe) skull from Zambia. The dates for these specimens are not entirely satisfactory but the Omo skulls may date to about 130,000 years ago (Butzer *et al.* *Quaternaria* 11, 15; 1969) and Broken Hill to about 110,000 years ago (Bada *et al.* *Proc. natn. Acad. Sci. U.S.A.* 71, 914; 1974). Even less securely dated but

also somewhat morphologically intermediate between *H. erectus* and *H. sapiens* are skulls from Saldanha in South Africa, Bodo in Ethiopia and Ndutu in Tanzania. As a group, these skulls are very heterogeneous but it is undeniable that they demonstrate varying degrees of sapientisation of the underlying *H. erectus* template. Skulls like Bodo and Ndutu, for example, occupy the more primitive *H. erectus* end of the morphological spectrum while others, such as L.H. 18 and Omo differ in fewer ways from modern *Homo sapiens*. Combined primitive and advanced characters are also seen in certain elements of the post-cranial skeleton such as the femora from the Broken Hill locality and from the Guomde Formation east of Lake Turkana (KNM-ER 999) (Kennedy, in preparation). This latter specimen may date to about 100,000 years ago (Fitch *et al.* *Nature* 252, 213; 1974).

The recognition that populations demonstrating a combination of *H. erectus* and *H. sapiens* features existed in Africa before 100,000 years ago poses some very intriguing questions. One such question concerns the source and nature of the evolutionary stimuli which led to the emergence of the modern species. Throughout its long evolutionary history, *H. erectus* demonstrated very little, if any, evidence of progressive morphological change. The earliest presently recognised members of the species, from the Lake Turkana area of Kenya and from Java, predate 1.5 million years. From this time through the Middle Pleistocene there is no clear evidence of progressive morphological advancement. According to some workers the first evidence of sapientisation occurred 300,000 to 400,000 years ago, during European Second Interglacial times. Others however, would maintain that the transition did not occur until about 100,000 years ago, during the Third Interglacial period. The group of archaic

hominids under discussion here, including L.H. 18, Omo and Broken Hill, may add slightly greater weight to a later transitional phase.

In any case, the long period of morphological stasis in *H. erectus* was ended by the appearance of individuals with significantly expanded, less angular skulls and thinner bone structure. It is of great interest that these more advanced morphological characters are not accompanied by obvious advances in stone tool technology, subsistence efficiency, settlement size or duration of occupation. This suite of traits is conventionally thought not to occur until the Upper Palaeolithic, around 35,000 years ago. Moreover, geographic range, already broad in *H. erectus* does not appear to change in the initial phases of sapientisation. What factors then were responsible for the evolutionary pressures and responses which ultimately led to the replacement of *H. erectus* by *H. sapiens*? Although the answer, in a very general way, may rest on increasing complexity of the behavioural repertoire and social organisation a more precise delineation of the process has not yet been accomplished.

A second question concerns the relationship of the very distinctive Neandertals both to these archaic sapients and to modern man. Controversies have continued for many years over whether anatomically modern man rapidly emerged from a single Neandertal/early sapient lineage in the Fourth Glacial or whether Neandertal and sapient lines diverged separately from a common ancestor sometime in the Middle Pleistocene. This hypothesis would view the two lines in coexistence until the extinction or assimilation of the Neandertals between 20,000 and 30,000 years ago. The Neandertal complex of characters first becomes visible in the fossil record in Europe during the Third Interglacial and is

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found widely distributed in Europe and southwest Asia during the early part of the Fourth Glacial. If the dates for the archaic sapients, such as L.H. 18, Omo and Broken Hill are accurate then this group preceeds the first known appearance of the Neandertals by a very short period of time. However, the highly distinctive suite of features which characterises the Neandertals is not present in the preserved remains of the archaic sapient group. Absent in the latter group are such Neandertal features as the occipital 'chignon', rounder (as opposed to vertical) parietals, very large cranial capacity and curved, robust long bones. Thus, whatever

the nature and origin of the Neandertal complex of characters, much of this morphology is not reflected in the archaic sapients which immediately pre-date them. From this evidence, can it be argued that a single lineage, anatomically progressive 110,000 to 130,000 years ago, rapidly evolved into the Neandertal populations (which are primitive in many features), then just as rapidly, later evolved into anatomically modern man? On the basis of the available evidence now emerging in Africa a dual lineage, extending back more than 100,000 years, seems a more likely hypothesis. Further confirmation from the fossil record, however, is required. □

considerably greater than the greatest alternating (peak) field applied. This seems to mean that the act of rotation, or 'tumbling', may considerably enhance the effect of a given peak alternating field, in demagnetising a given coercivity range within a sample. Effectively it makes the tumbling method a more powerful demagnetisation method than the stationary one.

Meanwhile, in this issue of *Nature* (page 48) A. Stephenson, of the University of Newcastle-upon-Tyne, reports his direct observations of the torque developed in a specimen which is rotating about an axis at right angles to an alternating field axis. The torque is quite appreciable, and is clearly related to the acquisition of rotational remanent magnetisation. Stephenson uses these results in support of a theory which relates rotational remanent magnetisation right back to the Barnett gyromagnetic effect; where a rock, rotating in the absence of a magnetic field, acquires a magnetisation caused by the alignment of internal magnetic moments along the axis of rotation (*Rev. Mod. Phys.* 7, 129; 1935).

It may well be that rotational remanent magnetisation is providing us with a means to investigate the Barnett effect, which has hitherto been very weak and difficult to deal with. According to Stephenson, the reason it has such a large effect in these experiments is that we are dealing not with the rate of rotation of the whole rock sample, but with the rate of rotation of the magnetic moment of a single magnetic grain when it is flipping between stable states. Since flip times are very brief, such rotational rates can be equivalent to 3×10^7 rotations per second greater. One could never hope to rotate any bulk material in the laboratory at such a speed, to enhance the Barnett effect.

However, that is not the end of the story. A second paper in this issue (page 49), again by Alan Stephenson, shows that curiously enough one does not even need the rotation of the specimen to produce what Stephenson terms a 'gyromagnetic remanent magnetisation'. He shows that one can achieve a similar magnetisation whenever one sense of flip predominates during alternating field demagnetisation, and that this will be observable whenever the magnetic material is sufficiently anisotropic. He has, with considerable ingenuity, manufactured an anisotropic specimen from stacked strips of magnetic recording tape. Sure enough, he is able, with no specimen rotation during an alternating field 'demagnetisation', to induce a remanence into the specimen which is clearly related to its anisotropy and which confirms his suggestion that gyromagnetism is the basis of the whole effect.

Stephenson's and Edwards' experiments are dealing with an effect which will touch upon several kinds of research. First, rotational remanent magnetisation provides us with a fairly easy means of studying gyromagnetism itself. At least

Gyromagnetisation: rocks in a spin

from R.L. Wilson

THE fascinating phenomenon of rotational remanent magnetisation (RRM) was unexpectedly discovered some 15 years ago as a result of geophysicists' attempts to interpret the natural permanent magnetisation of their rock samples. In the process of trying to separate the various magnetisations acquired by the rock during its lifetime by demagnetising the rock in an alternating magnetic field, it was found that slow rotation of a sample during alternating field demagnetisation could induce a remanence in the sample along the rotation axis even when no steady (for example geomagnetic) field was present. This phenomenon remains largely unexplained but several attempts at partial explanation are now emerging spurred on by novel and ingeniously derived experimental results.

The serendipitous discovery of RRM followed the use of a method of alternating field demagnetisation devised by Brynjolfsson and Sigurgeirsson at the University of Iceland (*Adv. in Phys.* 6, 247; 1957) to overcome a problem found in early attempts to demagnetise rocks by the application of decreasing alternating fields. It had been found that far from totally demagnetising the rock one actually replaced the original magnetisation with a new one due to the combination of the alternating field and the geomagnetic steady field in the laboratory.

One way of avoiding this was to cancel the local geomagnetic field in some way (As & Zijdeveld *Geophys. J.* 1, 308; 1958) but another was to 'tumble' the specimen to randomise the magnetisation due to the combined alternating and steady (geomagnetic) fields. Brynjolfsson and Sigurgeirsson developed a very clever method which involved rotating the sample

about a single axis defined by its direction of natural magnetisation and applying the demagnetising fields perpendicular to the rotation axis of the sample. As the two magnetisation components had different properties, the total natural magnetisation vector sometimes rotated through quite large angles during demagnetisation, and so the sample was at each stage reoriented in the apparatus to maintain the new direction of natural remanence aligned with the axis of sample rotation.

When we duplicated this apparatus in Liverpool, deciding (for some reason which no-one can now remember) that every other demagnetisation process would take place with the sample turned upside-down with respect to the rotation axis, we obtained to our astonishment field demagnetisation curves which clearly zigzagged about the usual smooth demagnetisation curve. The specimens were demagnetised less when oriented one way up than the other. The first observations of this effect had been published in 1967 by Doell and Cox (*Developments in Solid Earth Geophysics* 3; *Methods in Palaeomagnetism*, Elsevier, 1967) but they had used three-axis tumblers and their results were much more complicated and were not pursued. Eventually the idea of RRM was arrived at (Wilson & Lomax *Geophys. J.* 30, 295; 1972).

Once this phenomenon had been accepted the problem was to explain it and some recent experimental results are throwing light on this problem.

Interesting experiments by J. Edwards at Aberdeen University (*Geophys. J.* in the press) show that one can induce larger rotational remanent magnetisations by rotating the sample during only part of the demagnetisation process. He also shows that the slow rotation affects magnetic grains whose coercive forces are

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experimentally there is little difficulty in reproducing the effect, but the theoretical interpretation of the results may be fairly difficult. Second, gyroremanent magnetisation may help us to measure and study magnetic anisotropy from the point of view of the solid state physicist. Third, the measurement of magnetic anisotropy in rock samples may be carried out by this new method, and used to interpret the stress histories of rock samples during geological time; the long term stress undergone by rocks often causes recrystallisation, the crystals (including the magnetic crystals) becoming aligned in a manner which is related to the stress field present during recrystallisation. Fourth, the under-

standing of rotational remanent magnetisation may help palaeomagnetists to learn how to demagnetise their samples in a more effective way than hitherto. Stephenson's second paper shows that even a stationary specimen in zero steady field, if anisotropic, will acquire an induced remanence during 'demagnetisation'. The practical demagnetisation problem then becomes "How does one tumble a specimen optimally to avoid induced rotational remanence in isotropic samples, gyroremanence in anisotropic samples, and ordinary anhysteretic remanence due to imperfect cancellation of the geomagnetic field?". A problem of some complexity, although it could just have a simple answer.

ILPAT 1.2 is present in all the clones (as expected) but it is flanked by different DNA sequences in each case, implying genetic rearrangement. Moreover, the arrangement is different in all the clones studied not just in the clone expressing ILPAT 1.2.

In this respect the results differ from those of Hoeijmakers *et al.* (this issue, page 78). These workers have prepared cDNA clones from four variants of *T. brucei* in a very similar fashion. The genes corresponding to these variant-specific cDNA clones were then analysed in DNA digests from all four strains by the same Southern blot technique and again the results are consistent with genomic rearrangements. The important and unexplained difference between this and the previous work is that the rearrangement is found only in the homologous variant, that is, the one expressing the variant antigen corresponding to the cDNA probe.

In many ways this fits much better with existing ideas and models for such genetic rearrangements. Hoeijmakers *et al.* interpret their results to show that the same basic gene copy is present in all variants examined, but that the homologous variant seems to show an additional copy of the gene flanked by different DNA sequences and which must presumably be linked with expression of the antigen. The appearance of such an expression-linked copy could be explained by insertion of a transposable promoter next to one of the two copies of the gene which would be found in a diploid organism. Unfortunately information on the ploidy of the trypanosomes is still lacking. A second attractive possible mechanism is gene cassetteing, analogous to the interconversion of yeast mating types. The mating type locus of *S. cerevisiae* can exist in two interconvertible states, a or α , which control the ability of yeast cells to mate and sporulate. The a and α genes are distinct co-dominant entities, which can be regarded as non-homologous blocks of regulatory information. On the basis of genetic evidence it was thought that yeast cells contain a silent (unexpressed) copy of both the a and α information, and that the mating type is controlled by insertion of a copy (or cassette) of one or other of the genes into the mating type locus. Evidence compatible with such a model was presented in *Nature* recently (Hicks, Strathern & Klar *Nature* 282, 478; 1979; see *News & Views* 283, 811; 1980) and such a cassette hypothesis could also explain the data presented by Hoeijmakers *et al.* for the variant antigen genes.

If translocation, by whatever mechanism, is involved in antigenic variation, how can the non-random generation of variants be explained? Here the accumulating data on RNA sequences around splicing junctions may provide a clue. Lerner *et al.* (*Nature* 283, 220; 1980) have tabulated hnRNA sequences at 43 intron/exon splice junctions and although

How trypanosomes change coats

from Mervyn Turner

THE concept of the 'mobile' gene is fast becoming part of the dogma of molecular biology. Precise somatic genetic rearrangements are now being recognised at the basis of the expression of a growing number of genes. From being considered a genetic freak when they were discovered many years ago, translocatable elements in maize have now been joined by immunoglobulin genes and yeast mating type genes in eukaryotes and by the bacterial insertion elements and transposons. The newest additions to the list of mobile genes in eukaryotes are the genes specifying the wide repertoire of surface antigens in trypanosomes. It is now clear that the antigenic variation seen in these parasites of man and his domestic animals is a result of genetic rearrangement (Williams *et al.* *Nature* 282, 847; 1979; Hoeijmakers *et al.*, this issue of *Nature*, page 78).

Trypanosomiasis affects both man and domestic animals with profound economic consequences across a wide belt of Africa. The salivary trypanosomes are parasitic protozoa normally transmitted cyclically by tsetse flies. The trypanosome survives in the bloodstream of the mammalian host by periodically altering its antigenic profile so that the developing immune response of the host is abortive. The disease is therefore characterised by a relapsing parasitemia with each recrudescence representing the development of immunologically distinct variants. Clones of trypanosomes grown from a single organism can undergo variation, and the same antigens can often be detected in the bloodstream before and after the cyclical transmission, implying that a single organism contains all the necessary information to produce variants, and that variants do not arise by mutation. Although there seems to be no strict

sequence in which the different variants appear, there does seem to be a definable order of priority. The total number of variants which can be expressed by a single clone of trypanosomes is not known but it seems likely to run into hundreds. The variant antigens are glycoproteins of molecular weight about 60,000, which cover the entire surface of the trypanosome. Purified antigens show marked differences in isoelectric point and amino acid composition and the only reported protein sequence data show no homologies within the N-terminal 30 or so residues in four antigens isolated sequentially during the same rabbit infection.

The mechanism by which the information encoding these antigens is stored and expressed is obviously of interest, and two groups have now reported experiments bearing on this point. Williams, Young and Majiwa (*Nature* 282, 847; 1979) partially purified variant-specific mRNA from a clone of *Trypanosoma brucei*, in this case ILPAT 1.2, prepared double-stranded cDNA and inserted this into the plasmid vector pBR322. A DNA clone containing a 902 base pair coding sequence of variant-specific DNA corresponding to about half the antigen, was identified by hybrid-arrested translation. A probe prepared by nick translation of this sequence was then filter-hybridised to a Southern blot of restriction endonuclease fragments of nuclear DNA from four different clones of *T. brucei* including ILPAT 1.2. Three of these were interrelated, in that they were derived from different relapses within the same strain. The fourth was obtained from an unrelated strain.

In each case the probe hybridised strongly to one or more bands of nuclear DNA, the size of the hybridising fragments differing in each clone of *T. brucei*. The clear implication is that the gene encoding

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the sequences differ it is possible to deduce an idealised 'consensus sequence' using the most commonly found arrangements of bases. By analogy, translocation-specific DNA sequences may exist within the *T. brucei* variant antigen gene repertoire, one such sequence being associated with each gene. The degree of homology with the

ideal 'consensus sequence' could then determine the frequency with which translocation occurred at that particular variant. Clearly sequences from genomic DNA clones will be of immense value in any further studies on the mechanism by which this protozoan Generator of Diversity operates. □

More sensitive immunoassays

from Roger Ekins

A NEW immunological assay method, claimed to be considerably more sensitive than radioimmunoassay, has recently been reported. C. Harris and coworkers have developed an 'ultrasensitive enzymatic immunoassay' (USERIA) applied in this instance to detect cholera toxin and rotavirus (*Proc. natn. Acad. Sci. U.S.A.* **76**, 5336; 1979). Radioimmunoassay (RIA) and related methods have played a vital part in endocrinology, and subsequently in other areas of biomedical science since their original development some 20 years ago. Fundamental to these techniques was their exploitation both of the structural specificity characterising antibodies and other specific binding proteins and of the sensitivity of 'signal detection' implicit in the use of radioisotopic labels. These attributes, in combination, provided the basis of assay methods of sufficient sensitivity and specificity to identify and measure the often minute concentrations of many biologically active substances present in biological fluids.

These techniques introduced a new principle into microanalytical methodology: that is, the estimation of the amount or concentration of a substance of interest (the 'analyte') by observation of its distribution between 'reacted' and 'unreacted' moieties following its interaction with a strictly limited amount of the specific reagent (antibody, for example) used. This principle differs from that underlying most traditional analytical methods in biochemistry, in which the estimate of the concentration of an analyte relies on observation of the effect on (or

distribution of) a theoretically unlimited amount of a specific reagent following its interaction with the substance of interest for example, the measurement of a substance by exposure to light and observation of the extent of light conversion to fluorescent radiation. This principle underlies the immunoradiometric (IRMA) — 'radiolabelled antibody' — assay techniques developed a few years later in which excess of the specific reagent (radiolabelled antibody) is allowed to react with the analyte.

IRMA methods — like conventional RIA techniques — rely on a combination of the specificity of molecular recognition characteristic of antibodies (and other specific binding proteins) with the sensitivity inherent in radioisotopic measurement techniques. Nevertheless, although both IRMA and RIA methodologies possess many technical features in common, the contrasting analytical principles which they represent imply that they differ fundamentally both in their sensitivity and specificity characteristics, and in the overall incubation times required to carry out an individual measurement (Ekins in *Radioimmunoassay and Related Procedures in Medicine* **1**, 281, IAEA, Vienna, 1978). These distinctions arise largely as a result of the differing influence of the Law of Mass Action on the physicochemical reactions between analyte and specific reagent which constitute the basis of both analytical approaches. In particular, the 'labelled reagent' methods (such as IRMA) offer potential sensitivities many orders of magnitude higher than equivalent 'labelled analyte' assay techniques (such as RIA), primarily because the sensitivity of methods using the latter approach is

essentially limited by the avidity of the specific reagent which reacts with the analyte, as revealed by the equilibrium constant of the reaction. This theoretical constraint does not apply to the sensitivity of 'labelled reagent' methods whose ultimate sensitivity limits are primarily imposed by classical considerations of signal-to-noise ratio, and implicitly by the 'specific activity' (observable events/unit time/unit mass) of the label used.

It is particularly in the context of 'labelled reagent' methodologies that labels other than radioisotopes are likely to offer significantly greater sensitivity and precision, together with absence of bias and shorter assay times. They may also circumvent the problems and hazards of handling radioisotopes which have frequently, though not always legitimately, been advanced as a reason for the ultimate abandonment of the current generation of radioassay methods.

Enzyme-linked immunosorbent assay (ELISA) methods — conceptually identical to IRMA techniques and differing only in their substitution of an enzyme for a radioisotopic label — provide an example of a non-isotopic labelled reagent method which has proved valuable, for example, in agriculture (for the identification of plant viruses) in tropical medicine, and in other biomedical contexts, particularly those in which sophisticated instrumentation is out of place. Nevertheless, conventional ELISA methods have generally — though not invariably — failed to attain the high sensitivities which are implicit in the labelled reagent approach, essentially because the 'noise' (consequent upon 'non-specific' labelled antibody binding) is sufficiently large in relation to the 'signal' (generated by the specifically-bound antibody/enzyme-label complex) to preclude the detection of very low levels of individual analytes.

So the recent description, by Harris and coworkers (*Proc. natn. Acad. Sci. U.S.A. op. cit.*) of their ultrasensitive enzymatic radioimmunoassay is of considerable interest. Aside from the further terminological confusion inflicted on an already chaotic area of nomenclature, their methodological refinement is notable insofar as it utilises a radioactive substrate (^3H -AMP) for the quantitation of the specifically-

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100 years ago

Artificial Diamonds

An unusually large audience gathered at the Royal Society last Thursday to hear Mr. Hannay's account of his artificial diamonds.

The President, after inviting discussion of the paper by Messrs Hannay and Hogarth, observed that probably the large audience

had assembled more especially in consequence of the general interest attaching to the next paper on the artificial formation of the diamond, and he felt that the valuable investigation just detailed showed Mr Hannay to be a person worthy of attention when he claimed to have made even so startling a discovery as that on the face of this next communication. With regard to this the President observed that the attitude of science was always sceptical, and the Society would need ample proof that the metamorphosis of carbon into diamond had been really effected. . .

The President having called for any observations on the notice by Mr Hannay, Mr Maskelyne said that the present differed from the numerous announcements and

other communications that have been heretofore made to scientific societies at various times purporting to record the artificial production of the diamond in this, that here the product so claimed to have been manufactured is really diamond. He had himself proved this by the simple tests of the mineralogist. He had deeply abraded topaz and sapphire with a particle of the substance and abraded them with the greatest ease; the angle of the cleavages of a crystalline fragment sent him by Mr Hannay was the angle between faces of the regular octahedron, and he had burnt a small grain of the substance on a platinum foil with the characteristic glow of the diamond, and without its leaving a residue.

From *Nature* **21**, 4 March, 421; 1880.

bound enzyme-labelled antibody used in a conventional ELISA method. By this manoeuvre, Harris and his colleagues have succeeded in increasing the signal-to-noise ratio they observe compared with that attained using colorimetrically-based ELISA methods, and have thereby extended the detection limits of this technique to 10^{-16} g (that is 6×10^2 molecules) in the case of cholera toxin. In so doing, they have confirmed the proposition that labelled reagent techniques in general, and labelled antibody methods in particular, are potentially capable of attaining sensitivities many orders of magnitude higher than the 'labelled analyte' or 'saturation assay' methods exemplified by conventional RIA. Aside from the explicit advantages in terms of the reduction in detection limits that this advance offers, a corollary of the far greater sensitivity inherent in 'labelled reagent' methods is the significantly shorter assay incubation times that are required to achieve acceptably precise results.

The technique described by Harris and his colleagues — which might more accurately be described as 'enzyme-amplified' immunoradiometric assay — nevertheless constitutes a somewhat unwieldy approach to the attainment of high signal-to-noise ratios. Nor does it meet the environmental objections to the use of radioisotopes that are increasingly being levelled against both RIA and IRMA techniques. However other forms of label now being developed may offer the same high sensitivities while minimising the complexity of the chemical procedures or of the physical instrumentation required. Two approaches potentially capable of yielding assay sensitivities of the same order as that provided by USERIA rely on the use of chemiluminescent labels as described by Simpson and his colleagues (*Nature* 279, 646; 1979), and of fluorescent markers — particularly those such as the chelated rare earth fluorophores which, as discussed by Soini and Hammilä (*Clin. Chem.* 25/3, 353; 1979) can be readily distinguished from background fluorescence by time resolution techniques. Such alternative labelling methods are under active exploration in several centres. Combined with the exploitation of the *in vitro* hybridoma techniques of antibody production pioneered by Milstein and his colleagues at Cambridge (Milstein & Köhler in *Antibodies in human diagnosis and therapy*, 271, Raven Press, New York, 1977) with which large quantities of monospecific antibodies can be produced, the emergence of simple and reliable assay procedures far surpassing current RIA techniques in sensitivity, precision, speed, specificity, convenience and overall reliability is within sight. The implications of such a prospect in relation to biomedical research, to routine clinical diagnosis, and to related fields such as agriculture, are not difficult to visualise. □

Methane from ridges

from Peter J. Smith

ONE of the more important, but underpublicised, elements of progress in the Earth sciences during the past decade has been the growing appreciation of the crucial role played by hydrothermal fluids in the vicinity of oceanic ridges. Interest in the subject has widened recently, largely because of the physical implications; there can be little doubt, for example, that hydrothermal circulation can go far towards explaining the curious heat flow variations observed across active ridges. Some of the most fundamental consequences of such circulation, on the other hand, are bound to be chemical, for there are few better ways of inducing significant chemical reactions in rocks than to have them bathed in hot chemically-rich fluids.

It is the chemical side which has now attracted Welhan and Craig (*Geophys. Res. Lett.* 6, 829; 1979) — that is to say, the 'chemically-rich' aspect rather than the way the fluids react with the lithosphere. The particular observational site is the crest of the East Pacific rise at 21°N , where recently discovered hydrothermal vents are discharging turbid waters (some black, some white) at temperatures of up to 400°C . Three samples of these waters were found to be enriched (compared with the ambient seawater) in helium and radon, as are most known hydrothermal fluids whether they come from the Red Sea, the

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Guaymas Basin (Gulf of California) or other sections of the East Pacific rise. But Welhan and Craig find that the 21°N fluids are also highly enriched in hydrogen and methane — and that seems to be an original observation.

As the samples available to Welhan and Craig were contaminated by 'normal' seawater, numerical estimates are likely to be imperfect. Nevertheless, emission from the world oceanic ridge system, estimated by extrapolation from the East Pacific rise data, amounts to $1.3 \times 10^9 \text{ m}^3$ of hydrogen and $1.6 \times 10^8 \text{ m}^3$ of methane a year. These are, of course, unexpectedly large figures. The mean concentration of deep-ocean methane, for example, is only about $5 \times 10^{-6} \text{ ml kg}^{-1}$, which means that it can all be replaced by new methane from the ridges within a mere 33 years.

Where does the ridge methane come from? Is it, as is the helium, a mantle constituent brought to the crust in the magma rising at oceanic ridges, or is it produced in a reaction between the risen magma and the surrounding seawater? For the time being such questions remain unanswered. In raising them, however, it is curious that Welhan and Craig do not even mention Gold's recent hypothesis to the effect that the outgassing of methane present in the original Earth is still continuing (*J. Petrol. Geol.*, 1, 3; 1979, but given considerable publicity during 1978). Whatever one might think of Gold's hypothesis on the purely intuitive level, it is important that it should be measured against whatever data can be obtained.

Gondwanaland revisited

from Don Tarling

THE Fifth Gondwana Symposium* marked the coming of age of Gondwanaland 16 years after the inception of these symposia. There is now a clear consensus about the final configuration of this supercontinent some 180 million years ago. The unity of South America and Africa as 'western' Gondwanaland and of India, Australia and Antarctica as 'eastern' Gondwanaland has been widely accepted, but the fit of these two pieces has been controversial. New oceanic magnetic anomaly data now provide the required constraints (M.J. de Wit, Witwatersrand; M.W. McElhinny, Canberra; B.J.J. Embleton, CSIRO, Sydney; I. Dalziel, Santa Barbara).

The reconstruction was developed at a workshop in Johannesburg in July 1979 and is broadly similar to that of A.G. Smith & A. Hallam (*Nature* 225, 138; 1970) but

differs in several important respects. 'Eastern' Gondwanaland has been moved much further away from southeastern Africa, leaving room for the Mozambique Ridge, the Agulhas and Falkland Plateaux and so on. Malagasy, although still northerly, is also further from the East African coastline, although possibly not far enough to satisfy me.

The new reconstruction, as do all its predecessors, creates major difficulties in western Antarctica. It is clear that the Antarctic Peninsular existed in the Triassic-Jurassic so it can neither overlap the Falkland Plateau nor run parallel to southern South America (Dalziel). The 'solution' is, for the time being, to break up western Antarctica into discrete blocks that rotated during the Mesozoic evolution of this region. The structural grain of the Ellesworth Mountains is anomalous compared with the rest of Antarctica, but the evidence for its anticlockwise rotation is still largely based on the geometric requirements of the reconstruction itself. However, some palaeomagnetic evidence (K.S. Kellogg, US Geological Survey, Denver) now seems to be consistent with this interpretation. It is intended to use this fit as the base for a geological, geochrono-

*Held in Wellington, New Zealand on 11-16 February, 1980. The Proceedings will be published by Balkema Press at the end of 1980.

logical and resource geology map to be published at 1:10,000,000 in 1981 and de Wit requested data to assist in this. Such a compilation will allow further testing of the reconstruction but it is unlikely that location of the major blocks will be in error by more than a few 100 km. The importance of this map is thus to highlight areas for future research.

A major consideration was the extent of Gondwanaland. Nepal (C.J. Klootwyk, Canberra), Iran (H. Wensink, Utrecht) and Thailand (S. Bunopas & P. Vella, Wellington) were formerly parts of Gondwanaland and there is evidence that much of China (J.B. Waterhouse, Queensland; P. Tasch, Wichita) and possibly some central Asian blocks, with parts of southern Europe (D.H. Tarling, Newcastle upon Tyne), also formed parts of this supercontinent at various times, the degree of floral and faunal interchange between the Gondwanan and Laurasian continents having clearly altered as both continents evolved. The distribution of dinosaurs seems to indicate that Afro-

Indian links persisted well into Late Cretaceous times (E.H. Colbert, Flagstaff) although it is not clear if such links were provided by the Laccadive-Mascarene Ridge or whether access was along the Iranian-Afghanistan coastlines. Palaeobiogeographic provinces formed a significant part of the meeting, with the key glossopterid flora now being divisible into six stratigraphic units that have, so far, been correlated between India and Australia (J.F. Rigby, Geological Survey, Queensland; S.C. Shah, Geological Survey, Calcutta). The presentation of major advances in palynological correlations were predictable, but nonetheless of critical value for the Carbo-Permian (E.M. Truswell, BMR, Canberra) and Permo-Triassic (J. Anderson, Witwatersrand). Although this work solves many problems it also raises others; it destroys the previous, elegantly simple concept of a Gondwanaland drifting through polar regions and hence being glaciated at successive periods in different areas. The age of these ice sheet deposits are almost entirely Sakmarian (Lower Permian) and require much more complex explanations (D.H. Tarling in *Climatic Change*, (Ed. Gribbin) Cambridge University Press, 1978). □

vibrations with zero momentum parallel to the surface, as in infrared studies.

The short range interactions are much weaker but their effects can be separated because they can transfer large amounts of momentum to the electron (Ho, Willis & Plummer *Phys. Rev. Lett.* **40**, 1463; 1978). Tuning the spectrometer to the non-specular electrons immediately separates these processes, but at the price of requiring much greater sensitivity. We immediately gain two advantages: the need for a dipole moment is removed; and vibrations with non-zero parallel momentum can be seen. There is a further less obvious gain in information. The matrix element for the scattering involves an incident-wave momentum k_i an exit wave momentum k_f and the displacement of the j th atom, C_j , caused by the vibration:

$$\sum_j \int \exp\{i(\mathbf{k}_i \cdot \mathbf{r} - \mathbf{k}_f \cdot \mathbf{r})\} C_j \cdot \nabla V_j(\mathbf{r} - \mathbf{R}_j) d^3r \quad (1)$$

where V_j is the potential of the j th atom, assumed localised near \mathbf{R}_j . If we were investigating the modes of a molecule, it would be useful to know how C_j varied within the molecule. Equation (1) shows that the contribution of each atom to the matrix element has a phase

$$\exp\{i(\mathbf{k}_i - \mathbf{k}_f) \cdot \mathbf{R}_j\}$$

therefore by measuring at different values of $\mathbf{k}_i - \mathbf{k}_f$ it will be possible to identify the individual components of the mode, provided only that we already know the mean positions \mathbf{R}_j of the atoms. That is, we know the phonon 'wavefunctions' as well as the energy levels.

There is an analogy with angle-resolved photoemission experiments in which the energy and momentum of an ejected photoelectron implies by conservation the energy and momentum of the original state of the electron before excitation. (Li, Tong & Mills *Phys. Rev.* in the press). Alternatively knowing both the original and final electron E and \mathbf{k} we can infer the q and ω of the photon. If we read phonon for photon we have the EELS experiment (whether it involves the loss or gain of a phonon). The analogy extends even further: equation (1) gives the matrix element for interaction with a phonon defining atomic displacements C_j . The analogous matrix element for photoemission is

$$\frac{1}{\omega_j} \int \exp\{i(\mathbf{k}_i \cdot \mathbf{r} - \mathbf{k}_f \cdot \mathbf{r})\} \mathbf{A}_j \cdot \nabla V_j(\mathbf{r} - \mathbf{R}_j) d^3r$$

where \mathbf{A}_j is the 'A vector' of the photon field at the j th atom. This close analogy should lead to rapid development of the associated theory of EELS which will be needed to realise the full potential information in the data □

Vibrations of atoms at surfaces

from John Pendry

THE vibrational properties of atoms and molecules adsorbed on surfaces are determined by the detailed interaction with the surface: whether a molecule dissociates, to what degree an atom is coordinated by surface atoms, whether new species are formed. Knowledge of the way atoms vibrate is an essential complement to our static picture of atomic arrangements. The traditional tool for studying vibrations is infrared absorption, which has yielded much information about vibrations but is limited by the need for the bond to have a strong polar element to be infrared active, and in the case of metal surfaces by the electromagnetic boundary condition which allows only a normal component of the electric field. Electron energy loss spectroscopy (EELS) offers an alternative means of studying vibrational frequencies which to some extent escapes from these restrictions (Ibach *Phys. Rev. Lett.* **24**, 1416; 1970).

Experiments make use of 1-10 eV electrons, which can be monochromated to a few meVs. This represents much worse resolution than infrared can provide and severely restricts the power of the technique. It is unlikely that electron

spectrometry can approach 1 meV resolution and so the technique is mainly concerned with studying strong bonds to relatively light atoms. Despite these considerable restrictions EELS is proving a useful technique which is continually developing both experimentally and theoretically.

There are two ways in which electrons interact with vibrating atoms: through long range and short range forces. If the vibration has an associated dipole moment, an electric field extends far from the surface and operates on an approaching or receding electron for a relatively long period. If a dipole moment is present, this is the dominant mode for inelastic scattering. (Evans & Mills *Phys. Rev.* **B7**, 853; 1973). Because the forces are long range the electron loses energy but remains undeviated in direction. A specular reflection at the surface turns the electron around into an analyser which separates the inelastic component. Until recently this has been the predominant mode of operation of EELS experiments. The requirement of a dipole moment imposes some of the restrictions of infrared spectroscopy and the lack of any substantial momentum transfer to the electron in the inelastic collision limits the information that can be obtained to

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REVIEW ARTICLE

The role of hormone receptors and GTP-regulatory proteins in membrane transduction

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Cell membrane receptors for hormones and neurotransmitters form oligomeric complexes with GTP-regulatory proteins and inhibit the latter from reacting with GTP. Hormones and neurotransmitters act by releasing the inhibitory constraints imposed by the receptors, thus allowing the GTP-regulatory proteins to interact with and control the activity of enzymes such as adenylate cyclase. This theory may apply generally to membrane signal transduction involving surface receptors.

ADENYLATE CYCLASE, the enzyme that produces cyclic AMP, is part of a complex regulatory system that mediates the actions of hormones and neurotransmitters on their target cells. Structured within the lipid framework of the cell membrane, the enzyme system is composed of at least three classes of components (Fig. 1). Located at the outer membrane surface is the receptor (R) component containing a specific site for binding of hormones and neurotransmitters. At the inner face of the membrane are the catalytic unit (C) and the nucleotide regulatory component (N). The latter contains site(s) for binding GTP and is responsible for mediating the effects of GTP and the various hormones on the activity of C¹. Two types of N units have been distinguished functionally. One mediates stimulation (termed N_s), the other inhibition (N_i) of the adenylate cyclase activity by GTP. As discussed below, each type seems to be linked to separate classes of receptors for hormones and neurotransmitters.

Here I present a theoretical framework for the role of hormone receptors and N units in regulating adenylate cyclase activity. In essence, the theory suggests that the N and R units normally exist separately from C as aggregates or oligomers of an RN complex. In this complex, R inhibits interaction of N with GTP. Hormone binding to R triggers release of the inhibitory constraints imposed on N with resultant enhanced reaction with GTP, followed by breakdown of the oligomers to a monomeric RN complex. The latter reacts with C to form the holoenzyme structure depicted in Fig. 1. Depending on the type of R and N unit attached to C, the holoenzyme exhibits either increased or decreased production of cyclic AMP. In developing this theory, I review evidence for the existence of N_s and N_i and their complexes with R and C, describe the properties of the various components, give recent evidence that RN oligomers exist and discuss the possibility that the theory may be generalised to include R and N units that regulate membrane transduction processes other than adenylate cyclase.

The role of N_s in activation of adenylate cyclase

Before the discovery that GTP is the essential activator of adenylate cyclase² and that hormones enhance the nucleotide's action³, it was thought that hormone receptors interacted directly with the catalytic unit and that fluoride ion, a non-physiological activator, affected the catalytic unit directly⁴. It is now clear that the actions of both hormones (through their

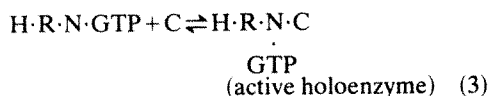
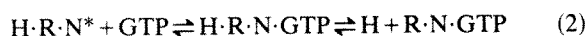
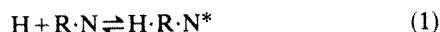
specific receptors) and fluoride ion are mediated through proteins that bind GTP. First identified^{5,6} with GTP-photoaffinity analogues as a heat-stable 42,000 molecular weight protein in detergent extracts of avian erythrocytes, the protein (N_s) has been shown to exist in a variety of cell membranes. Cholera toxin has been particularly valuable for identifying N_s. Long known to stimulate the production of cyclic AMP in animal cells⁷⁻⁹, the toxin potentiates the activating effects of GTP even in the absence of hormones and affects other characteristics of the enzyme that suggest that N_s is its primary site of action¹⁰⁻¹⁴. This was firmly established when it was found that the toxin, which contains in its A₁ subunit an ADP-ribosylating activity, preferentially labels the 42,000-MW protein in the presence of ³²P-NAD (refs 15-17). Cells deficient in N_s by functional criteria also lack the toxin-labelled protein; addition of detergent extracts of membranes containing N_s reconstitutes the ability of hormones, guanine nucleotides, fluoride ion and cholera toxin to stimulate cyclase activity in membranes from cells genetically deficient in N_s but otherwise containing hormone receptors and adenylate cyclase^{18,19}. Thus, judged from several standpoints, N_s is an essential component in the activation of adenylate cyclase. Although its structure remains unknown, N_s must have highly conserved recognition sites that allow it to activate C derived from a variety of cells and to 'couple' with the several types of receptors that mediate the stimulatory actions of hormones.

Table 1 lists a few of the properties of N_s and C when separate and combined (combinations with R are discussed below). C uses MnATP preferentially as substrate^{20,21}. Only when combined with N_s does C use the natural substrate, MgATP, as effectively as MnATP¹². Association of N_s with C is reversible and is driven by the binding of guanine nucleotides to N_s (refs 6, 22).

Properties and role of RN complexes

In classical theories of hormone action, the binding event leads the receptor to adopt a structure that is favourable for action. The relationship between hormone binding (K_D) and action (K_{act}) on adenylate cyclase systems is complicated by the fact that two ligands, hormone and GTP, are required for action². This complexity is exemplified by studies of the glucagon-sensitive cyclase system in liver membranes²³⁻²⁵. Direct binding studies with labelled glucagon revealed that GTP at concentrations required for activation of adenylate cyclase in the

presence of hormone, converted 90% of the receptors to a state with a higher K_D than K_{act} ; the remaining 10% displayed both the kinetic and thermodynamic properties commensurate with hormonal activation of the enzyme. A similar distribution of glucagon receptor states is seen in intact hepatocytes²⁶ which presumably contain sufficient GTP to interact with N_s at the internal face of the cell membrane. A plausible explanation for the effect of GTP on K_{act} and K_D is the following set of reactions (modified from ref. 27)



in which hormone binding and action are initiated at step (1) with resultant formation of an 'activated' state of $N(N^*)$. Reaction of the latter with GTP (step 2) leads to a complex ($H \cdot R \cdot N \cdot GTP$) which preferentially couples with C to form the activated holoenzyme. The negative heterotropic effects of GTP (decreased hormone binding) derive from a lower-affinity form of RN when occupied by GTP and not complexed with C (step 2). Thus, association of the macromolecular components (R, N, C) enhances the binding affinity of the small ligands (hormone and GTP). In this 'uncoupled' equilibrium model, the final concentration of the activated holoenzyme is a function of the relative concentrations of both the macromolecular components and the small ligands. In the overall equilibrium, all RN complexes have equal potential to form complexes with C but the amount of RNC formed is limited by the concentration of C. In the case of the liver system, the latter may be 10% of R and N.

Evidence from β -adrenergic receptor systems supports the above reaction scheme. For example, agonists but not antagonists promote the negative heterotropic effects of GTP on catecholamine binding²⁸⁻³². This is consistent with an ordered reaction in which hormones promote interaction of N in the RN complex with GTP. Evidence that the negative heterotropic effects derive from the RN complex stems from findings that cells lacking C but containing N_s and R display the negative heterotropic effects of GTP on hormone binding²⁰. Furthermore, RN complexes have been isolated following detergent extraction of membranes and separation from C (or NC); such complexes show the effects of GTP on hormone binding seen with intact membranes^{33,34}. Membranes from cells genetically deficient in N_s fail to show not only responses to hormones and guanine nucleotides, but also heterotropic effects of GTP on

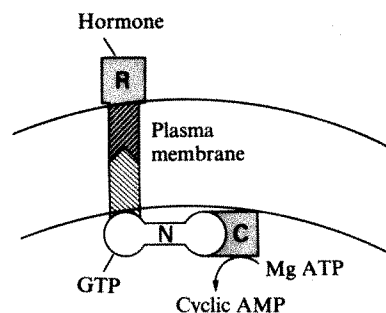


Fig. 1 Schematic representation of the components and organisation of the adenylate cyclase holoenzyme responsible for regulation by hormones and GTP. The receptor (R) is visualised as spanning the plasma membrane and having different segments (indicated by the shaded and cross-hatched areas) which have functions for binding of hormone, attachment to membrane and linkage with the nucleotide regulatory unit (N) that binds GTP. The N unit forms a bridge between R and the catalytic component (C) at the internal face of the membrane.

hormone binding to receptors; addition of extracts containing N_s to such membranes restores the ability of GTP to affect hormone binding³². Further evidence that the N_s unit responsible for activation of cyclase by guanine nucleotides, fluoride and cholera toxin is the same as that linked to R can be deduced from findings that cholera toxin affects the actions of GTP on both hormone binding and cyclase activity¹³. Accordingly, there is no need to invoke different regulatory N units linked to R and C, as has been suggested to explain differing properties of guanine nucleotide effects on hormone binding and adenylate cyclase activity^{27,33-37}. The differences can be explained by heterogeneous forms of N associated with the other components (RN, NC, RNC) having different properties with respect to affinities and actions of guanine nucleotides (Table 1).

The evidence cited above for the existence of RN complexes is based on the effects of GTP on hormone binding. On theoretical grounds, reciprocal effects of hormones on guanine nucleotide binding should also be observed. Recent findings^{38,39} that hormones promote the exchange of bound and free guanine nucleotides at the N_s unit provide further evidence that the R and N units are structurally linked. The functional consequences of this linkage on adenylate cyclase regulation are further discussed below.

It is evident that R units not associated with N units are nonfunctional with respect to adenylate cyclase activation by hormones; free R units in membranes have a lower affinity for agonists than do RN units (when unreacted with GTP)²⁸. Perhaps, free R units are formed from dissociation of RN units and are *en route* to endocytotic removal^{40,41}.

Table 2 lists receptors reported to show negative heterotropic effects of GTP on hormone binding and which are presumed, therefore, to represent RN complexes. Note that they fall into three categories: those involved in stimulation of adenylate cyclase (RN_s), those known to mediate inhibition of the enzyme (RN_i), and those which either do not interact with adenylate cyclase or whose function is unknown (RN_x).

Role of RN_i in the inhibition of adenylate cyclase by neurotransmitter and GTP

In contrast to its adenylate cyclase-activating role, GTP can inhibit some adenylate cyclase systems. First observed⁴² and characterised⁴³⁻⁴⁶ in rat adipocyte membranes, recent studies⁴⁷⁻⁴⁹ indicate that this process differs from the components that mediate stimulation by several lipolytic hormones in the same membranes. For example, adenosine promotes inhibition of adipocyte adenylate cyclase by GTP through processes which differ from the GTP-stimulatory process in their differential susceptibility to effects of sulphhydryl agents, proteases, divalent cations, sodium ions and mercurials.

Table 1 Properties of adenylate cyclase components

Components	Properties	Selected refs
C	Preferential reaction with MnATP as substrate	20, 21
NC	MgATP or MnATP as substrate when activated by Gpp(NH)p*, cholera toxin and NAD in presence of GTP, and by fluoride ion	16, 23
R	Low affinity for hormone agonists; no heterotropic effects of GTP	19
RN or (RN) ^p	GTP reduces affinity of R for hormone agonists. Hormones form tight-binding complex	24, 31, 35
RNC	Same as NC but responds to hormones and Gpp(NH)p; binds GDP tightly in absence of hormones. Hormones stimulate exchange of GDP and GTP at N site	38

*Gpp(NH)p is a GTP analogue that is not hydrolysed to GDP by GTPases in membranes.

The close functional linkage between the adenosine receptor (R -site⁵⁰) and N_i in the adipocyte suggests that there is an RN_i complex for adenosine in fat cells. It appears that RN_i and RN_s complexes in the fat cell interact with a common C unit⁴⁹. Opposing regulation by independent types of RN complexes (Fig. 2) may be widespread, particularly in cells that have adenylate cyclase systems governed by the endocrine and neuroendocrine systems. Receptors for such neurotransmitters as opiates, dopamine, catecholamines (α -adrenergic) and cholinergic agents (muscarinic) also mediate inhibition of adenylate cyclase by a GTP-dependent process⁵¹⁻⁵⁴. In common with the adenosine receptor linked to N_i in adipocytes, these receptors are sensitive to sodium ions. Interestingly, in the few cases examined, negative heterotropic effects of GTP on agonist binding are observed with the same membranes showing the GTP dependency of agonist inhibition of adenylate cyclase; sodium effects on agonist binding are also observed (Table 2). These findings suggest, by analogy with the role of RN_s complexes in the stimulation of adenylate cyclase, that RN_i complexes are involved in the inhibitory process.

The structure of RN

The technique of target size analysis has recently been used to examine the size of adenylate cyclase systems in various conditions in their membrane-bound form (for application and theory see ref. 55). Results obtained from liver⁵⁶, adipocytes and turkey erythrocyte membranes are shown in Table 3. The minimal size unit expressing activity is that obtained with MnATP as substrate and no activating ligands; it is presumed to represent C . The size increases significantly as N_sC is produced with pre-activation by guanine nucleotides or fluoride ion. Activation by hormones and GTP which should produce the holoenzyme (RNC), is accompanied by a further increment in size commensurate with this premise. In the case of the liver system, the sizes of the regulatory complexes (RN_s) were estimated from studies of the binding of labelled glucagon in the presence of GTP and from the 'ground-state' cyclase activity, that is the size of components present before coupling. Both studies indicate that the RN_s structure in the liver has a MW in the range $6-13 \times 10^5$, which is three to six times the estimated size of the RN unit associated with the holoenzyme. The conclusion from these findings is that RN_s exists as oligomers when not linked to the C unit. Note in Table 3 that the unit (presumably RN_i) responsible for inhibition of adipocyte adenylate cyclase by adenosine and GTP is significantly larger than the oligomeric RN_s unit that mediates the stimulatory effects of hormones and GTP on the same enzyme system. Although target analysis

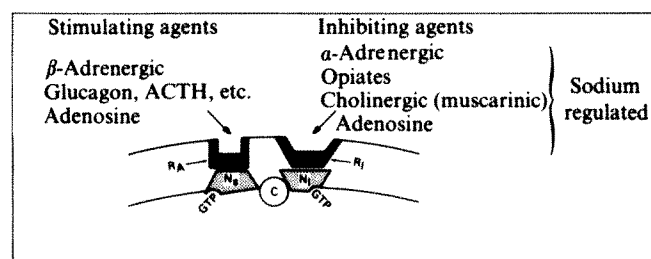


Fig. 2 Schematic representation of dual regulation of adenylate cyclase systems by stimulatory and inhibitory hormones and neurotransmitters. Depicted in the model are two classes of receptor (R) one (R_s) mediating hormone effects through stimulatory nucleotide regulatory units (N_s) and another (R_i) mediating inhibitory effects through linkage with an N_i unit that binds GTP and inhibits adenylate cyclase activity. The R_iN_i units require Na^+ for the effects of the various inhibitory agents on adenylate cyclase (C) activity.

cannot give the structure and composition of the target, it can be inferred from the size differences that the stimulatory and inhibitory processes reflect different structures that are multiples of the RN units comprising the holoenzyme.

Although the sizes of the adenylate cyclase system in turkey erythrocytes display increments with increasing regulatory complexity, this system does not show the oligomeric structures of RN_s ; the functional size remains identical before and after activation with catecholamines and guanine nucleotides. Possibly, R , N and C are already assembled in the holoenzyme structure due to prior activation of the enzyme system during isolation of the membranes. Pre-assembly may explain some notable differences in kinetic behaviour of the turkey and liver adenylate cyclase systems (discussed below). A pre-assembled unit is also consistent with the report⁵⁷ of a linear relationship (rather than the usual hyperbolic relationship) between hormonal activation and receptor occupation in the turkey system.

A model for hormone and GTP action on adenylate cyclase

The existence of oligomeric complexes of RN provides a structural basis for the uncoupled equilibrium reactions described above. For illustrative purposes, the model depicted in Fig. 3 shows a tetrameric structure of RN units existing in two

Table 2 Hormone receptors regulated by GTP (the RN complex)

Receptor type	Source	Type of N unit*	Comments	Selected refs
Glucagon	Rat liver	N_s	GTP = GDP; Gpp(NH)p and Gpp(CH) ₂ p less potent	24
Catecholamines (β -receptors)	Several cell types	N_s	Divalent cations promote binding of agonists; only agonist binding affected	28-32
Prostaglandin E	Thyroid, frog erythrocyte	N_s	Binding promoted by Ca^{2+} ions	98, 99
Dopamine	Corpus striatum	N_s, N_x	GTP = GDP > Gpp(NH)p; agonist specific	100, 101
Muscarinic	Canine and rat myocardium	N_i	Methacholine inhibits GTP effects on β -receptor; Na^+ probably affects binding	102, 103
	Neuroblastoma x glioma cells	N_i	Mg^{2+} enhances agonist binding	105
Catecholamines (α -receptors)	Brain	N_x	GTP = Gpp(NH)p > GDP; Na^+ affects agonist binding	106, 107
	Rat liver	N_x	Agonist specific	83
Angiotensin	Adrenal cortex	N_x	Agonist binding affected by Na^+ in same manner as by GTP	84
Opiates	Brain	N_x	Na^+ affects binding of agonists and antagonists. Two distinct opiate receptors	52, 104, 108

In all cases, addition of guanine nucleotides decreases binding of hormone or neurotransmitter to specific receptors in isolated membrane preparations.

* N_s is N unit linked to stimulation of adenylate cyclase; N_i affects inhibition of the enzyme; N_x is an N unit that is either not related to cyclase activity or has an undetermined relationship to a specific signal-processing system in the cell membrane.

configurations: an unoccupied structure (A) which favours binding of hormones but not GTP, and a hormone-induced or stabilised structure (B) which favours reaction of GTP with the N component (equivalent to N^* in the previous reaction scheme). Reaction with GTP results in dispersion of the oligomer to monomers (at this stage the negative heterotropic effects of GTP occur) which uniquely react with C to form the holoenzyme that converts MgATP to cyclic AMP. In broad terms, this 'disaggregation-coupling' model for hormone and GTP action can be likened to the manner by which cyclic AMP controls through its receptor the activity of protein kinase; the latter involves a change in the association of dimeric regulatory and catalytic subunits⁵⁸. In the case of hormone receptors associated with adenylate cyclase systems, their interaction with N units in the oligomeric state constrains the ability of N to react with GTP, thus preventing the formation of the 'active' form of the regulatory N unit (N_s or N_i). It follows that cyclase systems devoid of receptors linked to N should display high reactivity with GTP. This has been reported⁵⁹ recently with a strain of HeLa cells deficient in β -adrenergic receptors; when the same cells become enriched with receptors, catecholamines are required to restore the level of activity seen with GTP alone in receptor-deficient cells.

The disaggregation-coupling model has several other important features that serve to distinguish it from previous theories of hormone action⁶⁰⁻⁶². First, assuming that the oligomers of RN are unreactive with C, it provides a means for functional compartmentalisation of RN units from C and explains the observed dependency of the reaction on both hormone and GTP. Second, an oligomeric structure of RN allows for homotropic subunit interactions such that minimal occupation by hormones may cause near maximal production of the functional activating 'signal' (the RN-GTP monomer) in the presence of saturating concentrations of GTP; marked activation of adenylate cyclase with minimal occupation of receptors has been observed with several cyclase systems^{24,57,63,64}. Thus, although full occupation of receptor subunits may drive complete coupling of RN with C, coupling may occur even when these units are not occupied with hormone. Third, because the oligomeric RN structure is inert with respect to activation of adenylate cyclase, aggregation of uncoupled regulatory units is a means of 'turning off' the reaction cycle. How 'uncoupling-aggregation' is accomplished and what factors control this part of the cycle remain unknown. Possible candidates are cytosolic factors reported to affect adenylate cyclase activity⁶⁵⁻⁶⁹. Additionally, factors intrinsic to or associated with the plasma membrane may be essential in the aggregation-disaggregation cycle; these factors could include cytoskeletal elements⁷⁰ which may stabilise the aggregated units in the membrane; interestingly, agents that disrupt cytoskeletal structures have been reported^{71,72} to enhance cellular production of cyclic AMP in response to hormones.

Emphasis has been placed recently on regulation of hydrolysis of GTP by a GTPase putatively associated with the N_s unit^{14,73,74}

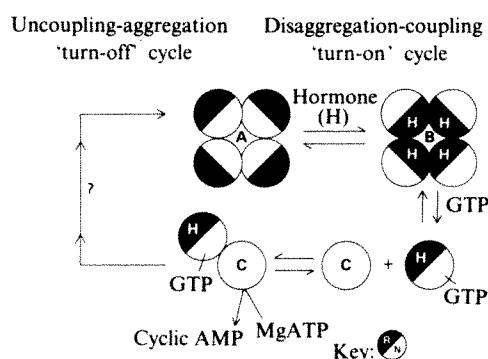


Fig. 3 A model for coupling of the receptor-nucleotide regulatory units (RN) to the catalytic unit (C) of adenylate cyclase and the role of hormone and GTP in this process. See text for detailed description.

Table 3 Functional sizes of adenylate cyclase components

Enzyme source	Components (MW $\times 10^5$)				
	RN_s	C	N_sC	RN_sC	R_iN_i
Rat liver: R = glucagon	6-13*	1.5	2.3	3.5	—
Fat cell (rat)					
R = catecholamine, ACTH	13	†	2.3	†	>13
R_i = adenosine					
Turkey erythrocyte	ND	0.9	1.8	2.5	—
R = catecholamine					

Sizes were determined by target size analysis using high energy irradiation. See ref. 56 for methods for determining size and rationale for assigning components to size. Data for fat cell and turkey erythrocyte data are unpublished. ND, Not detected.

* Estimated both from GTP-sensitive glucagon binding and adenylate cyclase activity of 'ground-state' enzyme.

† Not determined.

and the role of this regulation in turning-off the GTP-activation process. In turkey erythrocyte membranes, catecholamines and cholera toxin affect GTPase activity and GDP is a potent inhibitor that binds tightly to N_s (refs 38, 39). The differences in the kinetic characteristics of the liver cyclase system in its response to GTP and Gpp(NH)p are consistent with a GTPase being involved in its dynamic characteristics⁷⁵. However, GDP has been shown to stimulate liver cyclase activity in the presence of glucagon^{1,36,76}; stimulatory effects of GDP have been reported also in the presence of ACTH on the adipocyte adenylate cyclase system⁷⁷. In view of these findings it is not clear that a GTPase turn-off mechanism^{78,79} (which necessarily requires that GDP binds tightly and acts only as inhibitor) can adequately explain the dynamic properties of all adenylate cyclase systems. Perhaps the difference in the properties of the turkey, liver and adipocyte systems is related to the lack of an oligomeric structure of RN in isolated turkey erythrocyte membranes.

The topographical relationship between the RN oligomers and C in the plasma membrane and the role of membrane lipids in this relationship remain unknown. Studies with phospholipases have shown that hormone action on adenylate cyclase remains intact even after 85% of the membrane phospholipids have been digested; substantial loss in action occurs only when a fraction of the remaining phospholipids is hydrolysed⁸⁰⁻⁸². These findings could mean that RN oligomers and C are in close proximity and possibly in selective domains of interacting phospholipids; the bulk of the phospholipids do not seem to be involved in assembly of the holoenzyme.

Generalisations and problems

If the notion is accepted that the effects of GTP on hormone binding to receptors are due to disaggregation of oligomers of receptors linked to N units, then the theory cited above may apply generally to all hormone-regulated systems that illustrate these effects, even those N units (N_x) not linked to adenylate cyclase. The latter include such GTP-affected receptors as angiotensin receptors in the adrenal medulla⁸⁴ and α -adrenergic receptors in liver⁸³ (see Table 2). Recent findings⁸⁵ that insulin activates a cyclic AMP-independent protein kinase in sarcolemma membranes by a process regulated by GTP is an interesting example of a potential N_x -mediated process unrelated to adenylate cyclase.

The possibility that different N units mediate the actions of hormones raises interesting new questions. Can the properties of the same receptor be modified by interaction with different types of N units? What determines the interactions of receptors with a particular type of N unit? The pertinence of these questions is exemplified by reports that catecholamines, acting through α -adrenergic receptors (by pharmacological criteria), both stimulate⁸⁶ and inhibit^{51,54} cyclic AMP production, and induce effects unrelated to adenylate cyclase activity⁸³. Other hormones exerting multiple effects on membrane processes

include adenosine⁴⁸⁻⁵⁰, dopamine^{53,87-89}, opiates⁹⁰⁻⁹², vasopressin^{93,94} and serotonin⁹⁵. Pharmacological studies (specificity, potency, antagonist or hormone binding to receptors) alone may not identify the type of receptor mediating these processes. Heterotropic effects of GTP on agonist binding indicate linkage of receptors to an N unit but do not identify the type of N unit. I have noted in Table 2 that certain RN complexes are affected by sodium ions, others by divalent cations, and that guanine nucleotides have different potencies on agonist binding. Perhaps such differences can be used to classify the N unit associated with the receptor. Clearly, what is necessary in future research in this area is to develop assay methods that identify unequivocally each type of N unit. Testing of biological effects is not necessarily the means of determining the type of RN unit. A bizarre example of problems encountered is the action of cholecystokinin on pancreatic acinar cells⁹⁶. In the cell the hormone stimulates zymogen secretion, calcium release and cyclic GMP production, but not the production of cyclic AMP; after breakage of the cell, the hormone stimulates adenylate cyclase in a GTP-dependent fashion.

Although other explanations are possible, these findings raise the possibility that 'redistribution' of receptors and N units occurs on cell breakage owing to the breakdown of stationary domains that normally segregate these units in the cell. 'Lateral domain redistribution' might be a physiologically regulated process due either to changes in the structural relationship of the cytoskeleton to the ordered domains or to propagated disturbances in the membrane structure. A possible example of the

latter is the report⁹⁷ that catecholamines, acting through a β -adrenergic receptor, stimulate a phospholipid methylating enzyme by a process which is affected by GTP but which does not involve the production of cyclic AMP; associated with methylation is an apparent 'flip' of the internally methylated lipids to the outer face of the membrane and a change in lipid microviscosity. Pleiotropic effects of the hormone could thus be generated by localised changes in lipid structure being propagated laterally and modifying the postulated domain structures of the membrane. In any event, these findings question the commonly held view that β -adrenergic receptors are singularly linked to stimulation of adenylate cyclase. Moreover, the dependency of the hormone effect on GTP suggests again the versatility of the N units in mediating the actions of hormones.

In conclusion, the classical notion of receptor alone controlling the events related to hormone and neurotransmitter action on adenylate cyclase is no longer valid. The constraining role of hormone receptors postulated here differs from the role of the receptor postulated in other theories of hormone action. More importantly, the theory places in perspective the role of another set of regulatory proteins (the N units) hitherto given relatively scant attention, particularly with regard to their apparent multiple and fundamental roles in the regulation of membrane-associated processes. I hope this rather brief article will stimulate investigation of the new problems to be faced in ascertaining both the structures of the GTP-regulatory proteins and how they function in the transduction of hormone binding at cell membrane receptors into physiological action.

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ARTICLES

Cometary collisions on the Moon and Mercury

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Unusual swirl patterns of bright and dark material on the Moon and Mercury are proposed to be remnants of collisions with gas/dust-rich regions within a cometary coma. This interpretation provides important new clues for understanding cometary fine structure, impact effects of low-density material, and the origin of certain pronounced magnetic anomalies.

ALTHOUGH cometary impacts have long been assumed for inner Solar System bodies such as the Moon and Mercury^{1–3}, there has been little evidence for distinguishing such an event from a meteoroid impact. We propose that the enigmatic bright and dark swirls which cross portions of the lunar farside^{4,5} may be best explained by an impacting comet complex. The strong magnetisation of at least one swirl (Reiner γ) (ref. 6) and the close association of other swirls with lunar regions containing strong magnetic anomalies⁷ suggest that these features were magnetised in the impact. We argue that the swirls are young deposits, implying a recent cometary impact ($< 10^8$ yr ago) and ruling out an active lunar dynamo^{8–10} as the source of their magnetising field. Rather, this field may have been of cometary origin, perhaps amplified during the impact^{11,12}.

Bright/dark swirl patterns occur in three regions of the Moon. One of the best known examples is Reiner γ , which is near the western limb of the nearside (5° S, 60° W). The concentrations of patterns near Mare Marginis (15° N, 90° E) and Mare Ingenii (35° S, 180° E) are more impressive. Bright/dark swirls have not previously been studied in detail, but have generated several possible interpretations including nué ardente deposits¹³, antipodal effects of major basins⁴, volcanically derived sublimates⁴, secondary impact effects⁶, unusual secondary cratering phenomena associated with a cometary impact⁵, and selective preservation of albedo (crater rays) controlled by local enhancement of magnetic fields⁷. More detailed examination, however, reveals features that are suggestive of remnants of the impacting nuclear region of a comet.

Swirl patterns range in size from nearly 10 km to < 50 m across and form a variety of characteristic geometries: ribbon-like patterns, open loops and closed loops. As Fig. 1 shows, these patterns are commonly crossed by dark lanes. Both dark and bright patterns drape relief such as crater walls and rims and cross both the highlands and mare terrains (Fig. 2). At the highest resolutions available (~ 10 m), alteration of the surface (scouring) is not visible. Rather, the patterns represent diffuse brightening/darkening of unmodified terrains and commonly exhibit sharply defined boundaries over 50–100 m scales. In several examples, higher albedo regions correspond to sloped surfaces (for example, walls of degraded craters), whereas lower

albedo regions correspond to low-lying regions (for example, crater floor). Under high illumination, such patterns form a distinctive bright ring that strongly contrasts with other adjacent degraded craters. Ring patterns occur, however, in plains regions, not in association with changes in relief. Consequently, there may be several processes contributing to the formation of swirls.

Similar patterns are recognised on Mercury near latitude 20° N, 47° W and 4° N, 35° W. Because different phase angles and high-resolution images of these regions are unavailable, it is impossible to verify this identification in detail. Nevertheless, the bright loops and swirls noted at these locations are very similar to the gross patterns found on the Moon.

Although detailed photometry of the farside swirls is not available, several observations can be cited. Bright patterns occur on both dark (mare) and light (highland) surfaces. Apollo and Lunar Orbiter photographs under different illuminations and phase angles reveal that the bright patterns are strong forward reflectors (large phase angles) in contrast with most crater rays. This property, along with their characteristic pattern, make swirls easily identifiable. Dark lanes do not share this property, and under large phase angles, typically exhibit a reflectivity comparable to the surrounding terrains. Microdensitometer tracings reveal that the dark lanes are not simply an effect of contrast with the bright swirls, but exhibit a lower reflectivity under full illumination.

Longer wavelength measurements are available only for the nearside pattern, Reiner γ . Earth-based 70 cm (ref. 14) and 3.8 cm (ref. 15) radar data reveal no significant enhancements associated with Reiner γ . IR data during lunar eclipse¹⁶ reveal a possible 'cool region' that includes this pattern. Available data from the Apollo 17 Infrared Scanning Radiometer (ISR) provide much higher spatial resolutions (W. Mendell, personal communication) and also indicate no thermal enhancements. Consequently, Reiner γ exhibits a near-surface population of blocks resembling the average regolith. These data raise serious problems in the creation of a magnetic anomaly at this site, regardless of the proposed field source and remanence mechanism.

Swirl patterns are concentrated within two fan-shaped regions that open to the east (Fig. 3). Patterns near Mare Marginis converge near the 11-km diameter crater Goddard A, whereas patterns farther east converge near Mare Ingenii, perhaps

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towards a fresh 8-km crater on the wall of O'Day. The Reiner γ patterns may represent an eastern extension of the latter system.

The areal coverage, fine structure and shape of swirls change with distance from the western convergent zone. Near Mare Marginis, the greatest areal coverage by swirls occurs within ~ 300 km of Goddard A. Individual patterns typically cover 400 km^2 . Beyond 300 km east of Goddard A, swirls exhibit finer patterns each typically covering $< 100 \text{ km}^2$. In contrast, swirls abruptly disappear beyond 250 km west of Goddard A. Figure 3 also reveals that characteristic pattern shape changes in relation to Goddard A. Swirls are broad, arcuate loops roughly concentric with Goddard A to the west, but form parallel patterns within 400 km east of Goddard A. At greater distances, individual patterns form tightly closed loops, rings and narrow ribbons.

Although individual swirl patterns cross highland relief, there seems to be some topographic control at both fine and broad scales beyond 400 km from Goddard A. At fine scales (< 20 km), bright rings encircle a dark interior and commonly coincide with medium-size craters (~ 10 – 20 km diameter) having smoothly sloping walls. At broader scales, swirl patterns cluster within large craters and basins such as Fleming, Ingenii, Van de Graaff and Aitken.

We argue that swirl patterns are very young features. They clearly overlay the well preserved farside craters King and Necho, and appear to cross rays from Giordano Bruno. Crater statistics for King crater¹⁷ reveal a production population down to craters at least 20 m in diameter, thereby indicating a very young age for the swirl patterns ($< 10^8$ yr). The stratigraphic relation with Goddard A, however, is more complex. West of this crater, rays appear to roughen or 'feather' the bright swirl patches and cross dark lanes. Nevertheless, swirl patterns clearly superimpose ejecta deposits elsewhere. Such relations suggest swirl formation contemporary with Goddard A.

Recency of swirl formation is also indicated by the general absence of small, subsequent craters that excavate underlying contrasting material. Important exceptions west of King Crater exhibit a highly unusual morphology: bright-rayed and dark-rayed subdued dimple craters. Similarly, clusters of small, rayed craters superpose sections of the ejecta facies of Goddard A. These exceptions may be related to impacting material associated with swirl formation.

The crossing of relief, the absence of visible surface scouring and the photometric/IR properties suggest that the swirl patterns represent either a deposit or a physical alteration of the uppermost regolith layers. The distribution and geometry of patterns suggest a relation with the formation of the crater Goddard A and an exogenic origin that is unrelated to secondary cratering. These observational constraints are consistent, however, with highly dispersed material impacting the surface before and soon after Goddard A. Such a sequence is expected for a cometary impact where the tenuous inner coma should both precede and follow impact by the nucleus by 50–100 s. More specifically, the swirls may represent remnants of collisions by gas/dust-rich zones within the inner coma. These zones may be fine-scale counterparts to the streamers and knots commonly observed in the inner nuclear regions of comets as a result of sporadic explosions of material (ices, solids and gases) from the nucleus¹⁸.

The detailed structures of the inner coma and inner nucleus regions are, however, poorly understood. Number densities of the primary parent molecules of a cometary atmosphere¹⁹ suggest that the total coma mass (gas plus dust) within 200 km of the nucleus could range between 10^9 and 10^{10} g (This value assumes an isotropic coma where the number density varies as the inverse square of the distance from the nucleus. The parent components are assumed to approximate values for Comet K where $\text{H}_2\text{O}:\text{C}:\text{C}_2:\text{CN}:\text{CH}_3\text{CN}:\text{HCN}$ relative abundances are 17:6:2:0.5:1.0(?):2(?), respectively. The absolute number density of H_2O is taken as 2×10^{11} for a production rate of 3×10^{29} molecules s^{-1} and a lifetime of 2×10^4 s. It is further assumed that the gas/dust ratio is unity.). If the swirls cor-

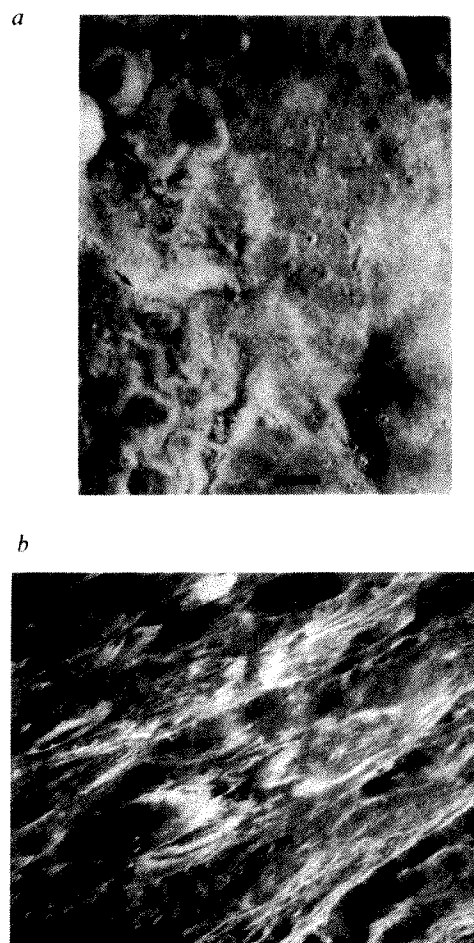


Fig. 1 *a*, Swirls 130 km west of King Crater at longitude 125° E, latitude 5° N showing characteristic looping pattern. Annulus of bright material at top coincides with a subdued crater. Arrow identifies ejecta from Necho crater that are crossed by dark lanes of the swirl patterns. Scale bar, 5 km. *b*, Swirl patterns overlapping rim of 23 km-diameter crater at longitude 110° E, latitude 11° N. Preservation on sloped surfaces suggests recent formation.

respond to gas/dust-rich zones, then this mass is not distributed isotropically, but is locally concentrated. Within 200 km of Goddard A, the total area covered by the swirls is $\sim 3,600 \text{ km}^2$, which represents nearly 10^5 km^3 . Thus, average mass densities in these zones may have ranged between 10^{-10} and $10^{-11} \text{ g cm}^{-3}$. Higher densities (10^{-8} – $10^{-9} \text{ g cm}^{-3}$) could be expected near the centre of major zones. Such values correspond to terrestrial atmospheric densities between altitudes of 90 and 120 km.

Although very tenuous, the derived gas densities are sufficient to heat, ablate and incinerate small meteors during entry into the Earth's atmosphere at velocities comparable to those expected, even for a low-speed cometary impact on the Moon ($\sim 20 \text{ km s}^{-1}$ ref. 4). This suggests that thermal alteration of the regolith may have occurred in the swirl areas and that the dynamic pressures (~ 0.1 bar) may have been sufficient to transport material on a micro-scale. Topographic control of reflected shock waves could locally enhance this process. In addition, if cometary dust particles are electrically charged and become confined within gas jets from the nucleus, their impact on the regolith at hypervelocities would generate an anomalous population of glasses and micrometre-sized iron particles. Both processes can alter the reflectance properties of the surface, which are sensitive to the mineralogy and petrology of the uppermost regolith²⁰. Consequently, the bright/dark swirl patterns are thought to represent high-velocity imprinting of the coma fine-structure by very fine-scale erosion/deposition and by the build-up of glassy materials through ablation of regolithic fine structure and impacts by cometary dust.

The recent discovery by Hood *et al.*⁶ that the nearside swirl feature Reiner γ coincides with a strong magnetic anomaly, and the suggestion that the swirls are generally in regions of strong remanent magnetic fields⁷ indicate that at least some of the lunar magnetic anomalies are of external origin, and are perhaps generated by cometary impact. Recent formation of these features precludes an origin by solar-wind stand off as proposed by Hood *et al.*⁶. Discussions of lunar anomalies must always treat two distinct problems: origin and strength of the magnetising field, and the nature of the remanence mechanism in the lunar rock which allowed a permanent magnetisation to be acquired^{21,22}. If remnant fields are associated with the swirls, only two courses are plausible for such a correlation: either the remanent fields predated the deposition, and merely served to guide the incoming coma material into a nonuniform pattern on the surface; or the field was present only during the emplacement of the deposits, during which time some remanence mechanism operated to generate the anomalies coincident with the swirls. In this case the magnetic fields are associated with the comet, or are generated during the impact¹².

The first hypothesis is untenable. To channel the plasma flow effectively, the remanent magnetic field B must exceed the value $B > (2\mu\rho)^{1/2}V$ at least one ion-gyro radius above the surface, where ρ and V are the mass density and flow velocity of the partially ionised coma gases, and μ_0 is the permeability of free space. Taking $V = 20 \text{ km s}^{-1}$ and $\rho = 10^{-10} \text{ g cm}^{-3}$ gives $B > 10^{-5} \text{ T} = 0.1 \text{ G}$. Since ρ may have been as large as $10^{-8} \text{ g cm}^{-3}$ in coma jets, lunar remanent fields of 0.1–1 G would have had to exist on large scales over the lunar surface at the time of the impact to channel the flow. Compression of the local field would ease this requirement somewhat, but not by more than an order of magnitude. As no evidence exists for broad-scale remanent field strengths of this magnitude on the moon, we reject this guided-plasma model.

Thus we argue that the remanent field now seen in swirls, such as Reiner γ , was acquired in the surface at the time of a comet

impact. It is likely that the magnetising field was intrinsic to the comet, perhaps generated in the nuclear region by a dynamo-like process²³ when the comet is close to the Sun ($< 1.5 \text{ AU}$) and thus very active, or simply due to the unipolar induction interaction of the coma with the solar magnetic field^{24–26}. In any case, the cometary field will probably be amplified as the coma is compressed against the lunar surface¹¹. However, the degree of amplification is not arbitrarily large, as current-driven plasma turbulence will create anomalous resistivity in the compressed region as the current density rises, and the electric fields will saturate. Although no data are available on field strengths in cometary comas, the poloidal field conversion model²⁵ gives values of 10^{-2} G , and allowing for the compression effects discussed by Gold and Soter¹¹, field strengths of 1–10 G should be present at the lunar surface during the impact for a considerable period. If the coma is $\sim 10^4 \text{ km}$ in size and hits the Moon at 20 km s^{-1} , large fields could be expected for times of the order of 10^3 s or more as the incoming tail continues to feed plasma and kinetic energy to the current systems flowing parallel to the lunar surface long after the nucleus has impacted. Apparently strong, relatively long-lived magnetic fields can be expected to be present at the lunar surface during such an event.

The critical problem in any impact-related generation of magnetic anomalies is the identification of a suitable remanence acquisition process. Shock remanence²⁷ is known to be effective in lunar surface materials when the shock pressures are in the range of 5–20 kbar. Such pressures are not likely to accompany swirl formation unless generated by individual microparticle impacts accompanying colliding gas/dust-rich regions. Moreover, these pressures could not be propagated beyond the rim region of the impact by the nucleus. Thermal remanence may be acquired if the jet of coma gas and dust impinging on the surface heats and alters the regolith in the presence of the ambient field. In this case each particle must cool through its Curie point while the field is still present. Such a process can occur if the impacting dust particles locally heat small volumes of

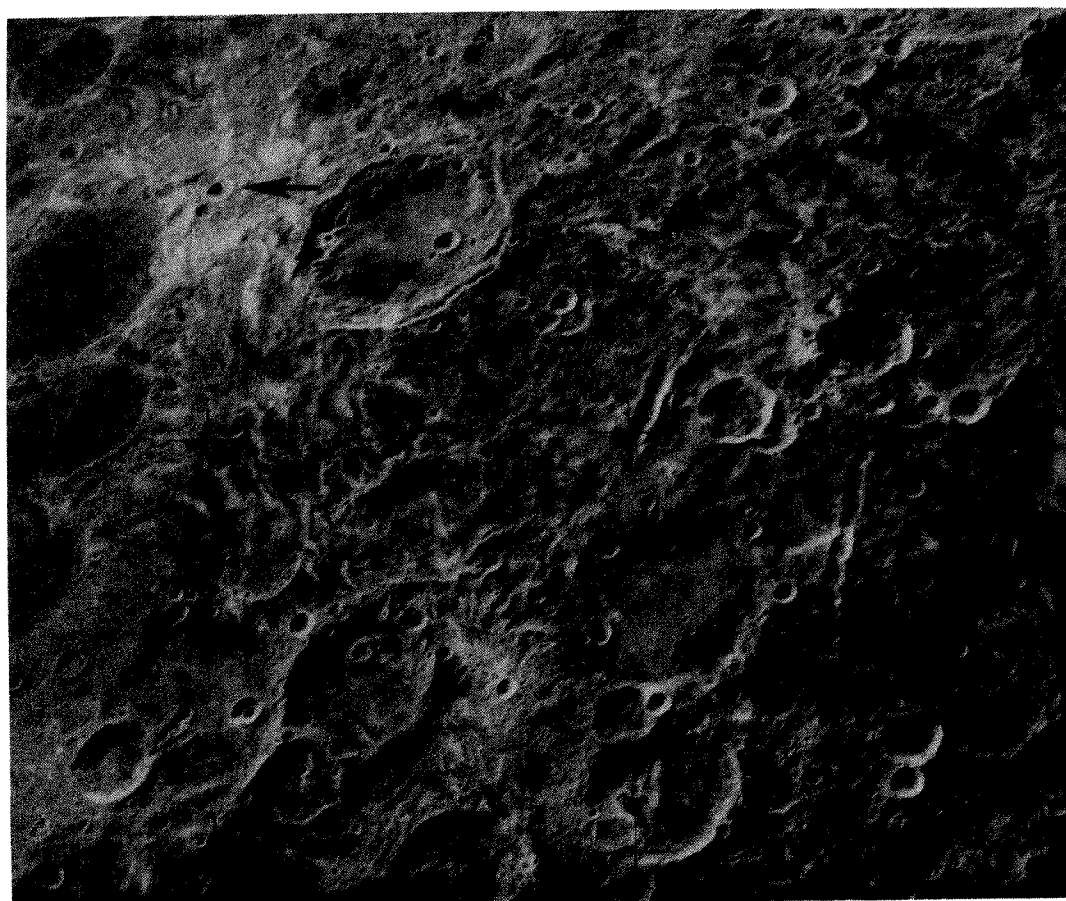


Fig. 2 Overview of region near Mare Marginis. Arrow identifies the crater Goddard A proposed to represent impact remnant of nucleus.

Fig. 3 *a*, Distribution of swirl patterns near Mare Marginis and farther east showing general concentration in a fan-shaped zone open to the east. Subarcuate patterns west of Goddard A (●) may represent hydrodynamic contact surface. Swirl patterns are proposed to be the result of fine-scale surface alteration by colliding gas/dust-rich regions within the inner coma. *b*, Distribution of swirl patterns near Mare Ingenii illustrating similar fan-shaped concentration. Reiner γ may be an eastern extension of this system.



soil, producing free iron droplets on a micrometre scale that cool in a fraction of a second and hence, acquire thermoremanence easily¹². However, it is difficult to explain specific features of the Reiner γ anomaly in this way. For example, the magnetisation direction for that swirl has a large component normal to the lunar surface⁶, whereas arguments based on a frozen-field MHD model suggests that the amplified cometary-field would be primarily tangent to the surface¹¹. However, the coma jet may have its own current system and magnetic field²³, in which case vertical fields may occur. A principal uncertainty is the inferred present surface strength of the remanent magnetic field at Reiner γ .

Using the data of Hood *et al.*⁶ and evidence that the swirl deposits are very thin (<1 m), we find a required regolith magnetisation I of $I > 0.8 \text{ e.m.u. g}^{-1}$ for a density of 2 g cm^{-3} , which exceeds by four orders of magnitude the stable component of remanence in lunar rocks, and by two orders the highest value of remanence observed in any lunar sample (G. W. Pearce, personal communication). This places severe constraints

on the composition of the regolith in the swirls. If we assume that the sampled lunar rocks constrain the remanence value in Reiner γ , then depths of alteration of 1 km or more are required to produce the Reiner γ magnetic anomaly. As the regolith depth of mare surfaces is <6 m, the remanence at Reiner γ must be much higher than any of the values yet found in the lunar samples. This demands either a very high free iron content, very high (perhaps saturation?) remanence in the iron grains, or both. The impacting coma gases (H_2O , CO_2 , and OH^-) also might alter the chemistry and mineralogy of the swirl areas, perhaps producing local concentrations of magnetite in the dark areas of the swirls.

If these interpretations are correct, then several statements can be made about the proposed comet. From scaling relations between crater diameter and impact energy²⁸, the size of Goddard A suggests a nucleus 200–500 m in diameter. Note that the resulting crater is not anomalous in appearance. The subarcuate patterns disappearing beyond 200 km west of Goddard A may correspond to a hydrodynamic contact surface,

which develops by collision of the sub-solar cometary atmosphere and the solar wind¹⁸. Broad swirls within 100 km of Goddard A correspond to the inner nuclear region of gases and dust, whereas the elongate and parallel patterns east of Goddard A delineate matter in the process of being swept into the flow patterns of the tail. Beyond 200 km from Goddard A, the remaining patterns indicate concentrations of gases controlled by magnetic field lines within the comet^{29,30} and/or by gases sublimating from ice bodies dislodged from the nucleus. The latter process could account for the unusual dimple-shaped bright and dark-rayed craters associated with certain deposits west of King crater. The general restriction of the swirl patterns within a fan-shaped zone reflects the projected form of the streamlined inner coma on the lunar globe. Clusters of small primary craters superposing the ejecta facies Goddard A and a few swirls suggest that a myriad of centimetre/metre-size meteoroids may have accompanied the event.

We take the correlation of a strong magnetic anomaly with one of the swirls, Reiner γ , as evidence for a strong magnetic field within the comet's coma. We suggest that the swirls which we have mapped on the lunar surface are generally magnetised due to thermoremanence or shock remanence (or both) in a highly altered lunar regolith, which has not been sampled by any lunar mission.

The distribution of swirls also may provide a clue to the direction of impact. The two major concentrations of swirls seem to be nearly symmetric about a line of latitude, thereby suggesting an orbit close to the ecliptic. A highly inclined orbit could result in a more asymmetric distribution. The east-open, fan-shaped envelope of deposits and the possible remnant of one impacting nucleus (Goddard A) suggest that the Moon must

have been in the waxing phase. Moreover, the relative timing between Goddard A and formation of the swirls requires that the tail of the comet was pointing away from the surface. From these considerations, the proposed comet would have been an incoming, prograde body. Such an orbit would reduce the relative impact velocity since the comet and the Earth-Moon system would be travelling in the same direction.

The excellent preservation of all swirl deposits, which appear to be surficial in character, may indicate formation by a single cometary system. A simple impact by one body does not seem possible owing to the separate Goddard A and Ingenii swirl concentrations. However, these patterns could result from a split cometary body, a common phenomenon¹⁸. Although not essential to our argument, Reiner γ also could be included in this system if it formed $3\frac{1}{2}$ days in advance of the Ingenii/Goddard A patterns. Rotation of the Moon during this time would geometrically allow the formation of Reiner γ beyond 180° from Goddard A.

Thus, it is plausible that a prograde, incoming, split comet system impacting the Moon between a waxing crescent and gibbous phases. Goddard A may represent the impact crater by a nucleus, whereas a small crater on the rim of O'Day may represent a second nucleus. The Reiner γ swirls could be part of the latter event, rather than a separate nuclear region. Although the most visible results are the swirl patterns, the unusual dark-rayed/bright-rayed dimple craters and a large number of small bright-rayed (non-secondary) craters near Mare Marginis may indicate an additional flux of small-size (<10 m) debris impacting nearly simultaneously.

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Sequence of the human insulin gene

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The human insulin gene contains two intervening sequences, one is within the region transcribed into the 5'-untranslated segment of the mRNA and the other interrupts the C-peptide encoding region. A comparison of the human with the rat insulin genes indicates potential regulatory regions in the DNA segment preceding the gene and suggests that the ancestral form of the insulin gene had two intervening sequences.

DIABETES MELLITUS is a complex disease in which the primary clinical symptom is abnormally high fasting plasma glucose levels. In its many forms it may affect as much as 5% of the human population, and its incidence appears to be increasing¹.

The hormone insulin, isolated in 1922 by Banting and Best², regulates normal glucose homeostasis and also has other phy-

siological effects. Insulin appears to exert its biological activities through interaction with a receptor present on the membrane of most, if not all, cells³. This leads to a pleiotropic effect on several physiological processes including uptake of various nutrients, and enhanced synthesis of glycogen, lipids, certain amino acids and proteins⁴. In addition, it promotes proliferation of many

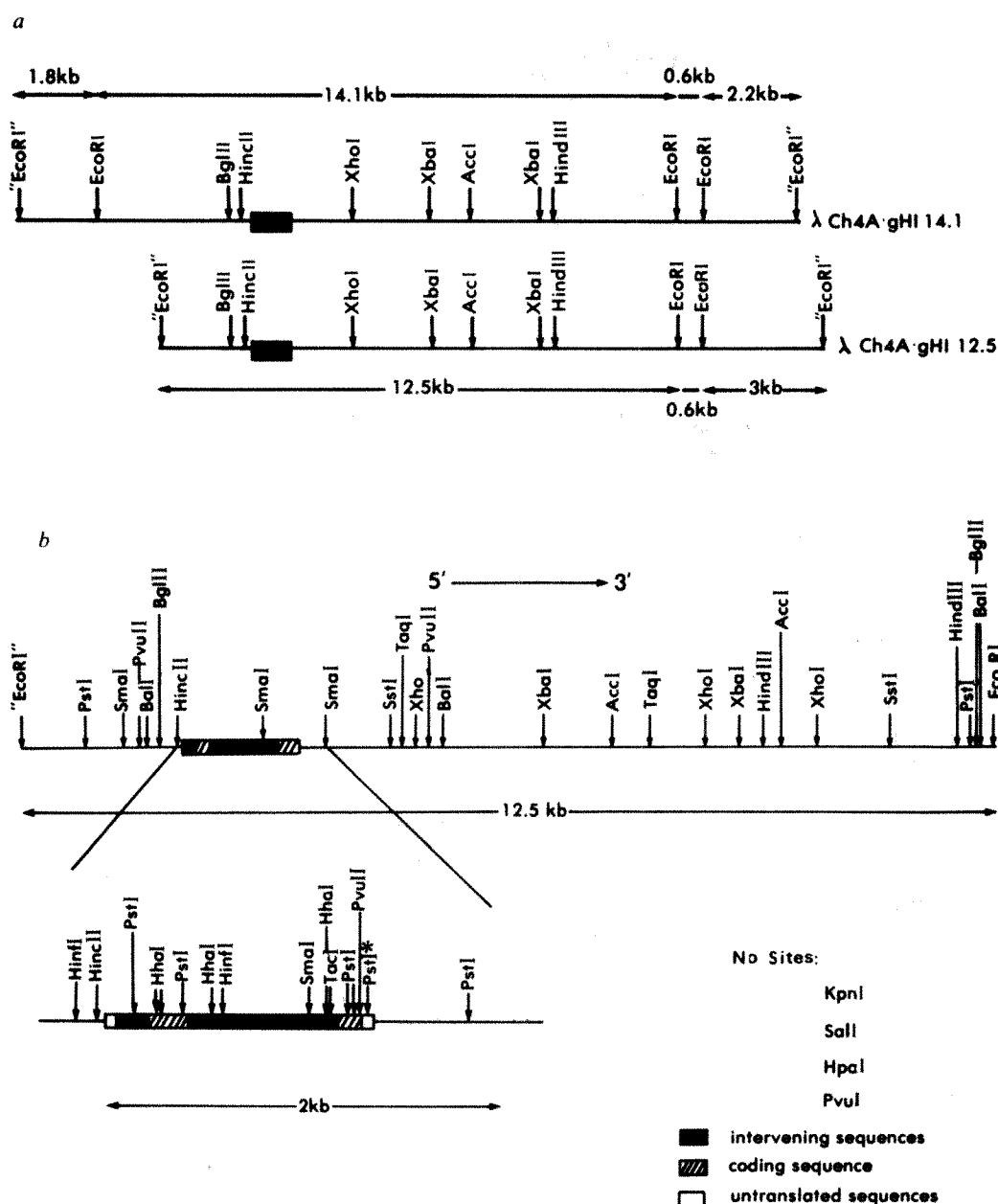


Fig. 1 a, Restriction maps of inserts of λ Charon 4A human insulin gene clones. A cloned library of approximately 20-kilobase fragments of human fetal liver DNA constructed as described by Lawn *et al.*²² was used. Plaques were screened for human insulin gene sequences as described previously¹⁹ by hybridisation with a nick-translated human insulin cDNA probe²¹. Phage containing the insulin gene were plaque purified and DNA was then prepared from phage grown in liquid culture. After digestion with *EcoRI* and agarose gel electrophoresis, the DNA fragments which contained insulin gene sequences were identified after hybridisation with human insulin cDNA²³. The order of the *EcoRI* fragments and the sites for other restriction enzymes within the human DNA inserts were determined by analysing multiple restriction enzyme digests¹⁹. The exact position of the insulin gene within the 12.5-kilobase (kb) *EcoRI* fragment of λ Charon 4A gHI 12.5, indicated by the box, was determined by more detailed restriction endonuclease mapping (b) and DNA sequencing. The position of the insulin gene in λ Charon 4A gHI 14.1 was inferred from the comparative restriction endonuclease analysis of the two clones. The *EcoRI* sites within quotation marks are those of the *EcoRI* linkers used in construction of the library and are adjacent to the λ arms. b, Partial restriction map of the human insulin gene and adjacent regions. The 12.5-kilobase insulin gene containing *EcoRI* fragment of λ Charon 4A gHI 12.5 was

subcloned into pBR322 (pGHI 12.5). The restriction sites were determined after single and multiple enzyme digests. The relative position of insulin coding sequences was determined by the hybridisation technique of Southern²³. The exact location and structure of the gene were determined by DNA sequencing. The orientation of the gene is indicated by the arrow.

animal cells in culture⁵. These processes may be mediated at least in part by an effect on the intracellular second messengers calcium and/or cyclic AMP⁶. Although administration of the hormone ameliorates the diabetic symptoms and allows a more or less normal life, diabetes and its complications are still the third leading cause of death in the US¹. The aetiology of diabetes is poorly understood; both environmental (dietary, viral and chemical) and genetic factors are involved^{1,7-9}.

Insulin has been isolated from a variety of vertebrate species^{10,11}. In all instances, the molecule is composed of two polypeptide chains (A and B) joined by disulphide linkages. In most species examined, there is only a single insulin and consequently, it is believed, a single gene. However, three species of rodents (laboratory rat, mouse, and spiny mouse) and two species of fish (tuna and toadfish) contain two distinct insulins^{11,12}, inferring the presence of two non-allelic insulin genes.

The immediate translation product of the insulin gene is a single polypeptide, preproinsulin, which contains a 'signal' peptide at the NH₂-terminus which facilitates the transit of the insulin precursor into the endoplasmic reticulum and is cleaved away during the process¹³⁻¹⁵. The resultant molecule, proinsulin, in which the peptides B and A are joined by the connecting C-peptide (NH₂-B-C-A-COOH), is the precursor of insulin¹⁶. The connecting peptide is believed to aid the formation of the appropriate conformation of the molecule and thus correct formation of the disulphide bridges. Consistent with this view, there is considerable variation in the structure of the C-peptide between species¹⁷.

Until recently there was no specific knowledge about insulin mRNA or the insulin gene. Using recombinant DNA methodology, we isolated a DNA complementary to the messenger RNA of rat insulin and determined its sequence¹⁸. The overall structure of rat preproinsulin was determined, and

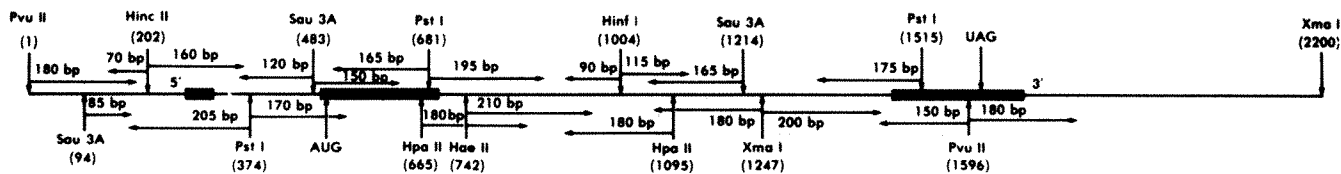


Fig. 2 Strategy for sequencing human insulin gene and adjacent regions. End (5')-labelled restriction fragments were sequenced by the procedure of Maxam and Gilbert²⁴. The cleavage products were separated by electrophoresis in 0.38-mm thick 10% and 15% polyacrylamide gels. The direction and number of bases determined from each labelled restriction site are indicated. The coding regions of the gene are indicated by the boxes. The orientation of the gene and positions of the translational start and stop are also indicated. The number in parentheses is the position of the restriction site (Fig. 3) used to initiate a particular sequencing run. bp, Base pairs.

in addition, certain unknown amino acid sequences were established. The cloned insulin cDNA was used as a hybridisation probe to isolate fragments of genomic DNA containing the insulin genes^{19,20}. The genes encoding rat insulins I and II each contain an intervening sequence of 119 base pairs in the 5'-untranslated portion of the mRNA. In addition the rat insulin II gene (but not the rat insulin I gene) contains an intervening sequence (499 base pairs) in the region of the DNA coding for the C-peptide²⁰. Again using the rat cDNA as a probe to screen bacterial colonies containing recombinant plasmids derived from human insulinoma mRNA, we isolated a cDNA clone encoding human preproinsulin²¹. The nucleotide sequence of the human insulin mRNA is quite similar to the rat insulin mRNA. The human insulin cDNA was used as a probe to screen in turn for insulin sequences in a library of human genomic DNA fragments. We report here the complete sequence of the human insulin gene and the immediately adjacent DNA areas, and compare it to the rat insulin I and II genes.

Isolation and restriction endonuclease digestion mapping

The human gene library in bacteriophage λ Charon 4A described by Lawn *et al.*²² was obtained from Dr T. Maniatis. The library was constructed by partial digestion of human fetal liver DNA with *Hae*III and *Alu*I restriction endonucleases, size fractionation, methylation with the *Eco*RI methylase, *Eco*RI oligonucleotide linker addition, and cloning in the λ vector, Charon 4A. We screened the recombinant phages for those containing human insulin gene sequences by hybridisation with a radiolabelled cloned cDNA fragment encoding human preproinsulin²¹. Two out of approximately 10^6 phages hybridised with the probe. These two clones were purified and the DNA characterised. One clone (gHI 12.5) contained *Eco*RI fragments of 0.6, 3.0 and 12.5 kilobases, the other (gHI 14.1) contained *Eco*RI fragments of 0.6, 1.8, 2.2 and 14.1 kilobases. The largest *Eco*RI fragment in each isolate hybridised with the cloned insulin cDNA. Since the library was constructed from human DNA fragments obtained by essentially random cleavage, the data suggested that the two clones represent overlapping fragments of human DNA. Further restriction mapping confirmed this view and allowed ordering of the *Eco*RI fragments (Fig. 1a).

The 12.5-kilobase *Eco*RI fragment of gHI 12.5 was subcloned in the plasmid pBR322. A detailed restriction map of this fragment was constructed (Fig. 1b). The restriction mapping experiments indicated that the gene was not co-linear with the mature mRNA, since the extent of the coding regions was greater than the 416 base pairs present in the cloned human insulin cDNA used as a probe. Furthermore, the enzyme *Sma*I generated two fragments of 900 and 1,500 base pairs which hybridised with the cDNA probe. Since no *Sma*I sites were predicted by the mRNA sequence²¹, it seemed likely that the gene possessed an intervening sequence containing an *Sma*I site. The pattern and sizes of the *Pst*I fragments hybridising with the insulin cDNA were also consistent with the presence of an intervening sequence within the gene (see later Fig. 5).

Sequence and general organisation

The complete nucleotide sequence of the cloned human insulin gene, gHI 12.5, was determined by the procedure of Maxam and Gilbert²⁴. The strategy used is presented in Fig. 2. Insofar as possible both strands were sequenced and all restriction

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CAGCTGTCAGCAGGACAGGTC1G6CCACCGGGCCCTGGTTAAGACTCTAATGACCCGCTGGTCCCTAGG
100
AAGAGGTGCGCAGCAGCAGGAGATCTTCCACAGACAGCAGCAGCAGGAAATGGCTCCGAAATTCGACG
150
CTCAGCCCCCAGCCATCTGCGCAGCCCCCCCCCCCCCAATGGCCAGCGCGGAGGGGTGACAGGTAG
200
GGGAGATGGGCTCTGAGAC(TATAAAG)CCAGCGGGGGCCAGCAGCCCTAGCCCTCCAGCAGCGCTG
250
CATCAGAAGAGGCCATCAAGCAG(GTCTGTCCAAAGGGCTTTCGCTCAGGTGGGCTCAGGGTCCAGGG
300
TGGCTGGACCCAGCGCCAGCTCTGCAGCAGGAGGACGTGGCTGGGCTGCTGAAGCATGTGGGGGTGAG
350
CCCAGGGGGCCCCAAGCAGGCGACCTGGCTTCAGCTGCCCTCAGCCCTGCCCTGCTCCAGC)ATCACCTG
400
500 Met Ala Leu Trp Met Arg Leu Leu Pro Leu Leu Ala Leu Leu Ala
TCCTTCTGCC ATG GCC CTG TGG ATG CGC CTC CTG CCC CTG CTG GCG CTG GGC
Leu Trp Gly Pro Asp Pro Ala Ala Ala Phe Val Asn Gln His Leu Cys Gly Ser
CTC TGG GGA CCT GAC CCA GCC GCA GGC TTT GTG AAC CAA CAC CTG TGC GGC TCA
His Leu Val Glu Ala Leu Tyr Leu Val Cys Gly Glu Arg Gly Phe Thr
CAC CTG GTG GAA GCT CTC TAC CTA GTG TGC GGG GAA CGA GGC TTC TTC TAC ACA
Pro Lys Thr Arg Arg Glu Ala Glu Asp Leu Gln V
CCC AAG ACC CGC CGG GAG GCA GAG GAC CTG CAG G (GTGAGCCACCGCCCATTCGCTGC
700
CCCTGGCGCGCCAGCCACCCCTGCTGCTGGCGCTCCACCCAGCATGGGCAAGGGGCGAGGAGCT
750
GCCACCCAGCAGGGGGTCAGGTGCACTTTTAAAGAAAGTCTCTCTGCTGCTCCTTAAAGTGACCAAG
800
CTCCCTGTGGCCAGTCAGAACTTCAGCTGAGGACGGTGTGGCTTGGGAGCCCGAGATACATCAGAG
850
GGTGGGACAGCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT
900
ATGAGCCGAGATTCAAGTGTTTTGTAAAGTAAAGTCTTGGTGACCAAGGGGTACAGGGTGGCCACGCTG
950
CCTGCTCTGGGCGAACACCCATCAGCCGAGGGAGGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCTGGCT
1000
TGTGCGCAGCTTCACGGCAGCTCCATAGTCAGGAGATGGGGAAGATGCTGGGAGAGGCTGGGAGAGAG
1050
TACTGGGATCAGCTGTTCAGGCCTCCACTGTGACGCTGCCCGGGGGGGGAGAGGAGGTGGGACATGTGG
1100
GCGTGTGGGGCTGTAGGTCCACACCCAGTGTGGGTGACCCCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT
1150
ATGGGTGGAGTGGCACTAGGGCTGGCGGGAGGGCGGACCTGTGTCTCTCTCTCTCTCTCTCTCTCTCTGT
1200
CCCTCTGCTCTGGCGCTGTCTCGGAACCTGCTCTGGCGGACAGTCTCTGAGC)
1250
Glu Leu Gly Gly Gly Pro Gly Ala Gly Ser Leu Gln Pro Leu Ala Leu Gly Gly
GAG CTG GGC GGG GGC CCT GGT GCA GGC AGC CTG CAG CCC TTG GCC CTG GAG GGG
Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys Thr Ser Ile Cys Ser Leu
TCC CTG CAG AAG CGT GGC ATT GTG GAA CAA TGC TGT ACC AGC ATC TGC TCC CTC
Tyr Gln Leu Glu Asn Tyr Cys Asn Asn
TAC CAG CTG GAG AAC TAC TGC AAC TAG ACGAGCTTCAGGACAGCCACACCCCGCGCT
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CCTCGACGAGAGAGATGGAATAAGGCCCTTAAGCAGC CCTCTGTGTCCTCTCTCTCTCTCTCTCTCTCT
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Fig. 3 Sequence of human insulin gene and adjacent regions. The sequence of the human insulin gene and its translation into preproinsulin in one frame is presented. The 'Hogness' sequence, TATAAAG, is boxed. The putative 5'-end of the mRNA (capping site) is indicated by an asterisk, while the 3'-end of the mRNA (poly(A) addition site), is indicated by an arrow. The intervening sequences are indicated in parentheses and were positioned by comparison with the cDNA sequence of human insulin mRNA²¹ and the rat insulin genes^{19,20} and by using the GT/AG rule (see text). The last digit of the number is placed over the corresponding base.

cleavage sites were confirmed by sequence analysis with the exception of the *Xma*I site at position 1,247 (*Xma*I is an isoschizomer of *Sma*I) and the *Pvu*II site at position 1,596. This *Pvu*II site is within the coding region of the gene and the sequences on either side are co-linear with the mRNA sequence as determined from the cDNA clone. From restriction mapping experiments we are confident that no small fragments were missed. The sequence of the segment including the gene and adjacent regions (262 base pairs before and 97 base pairs after the gene) is presented in Fig. 3.

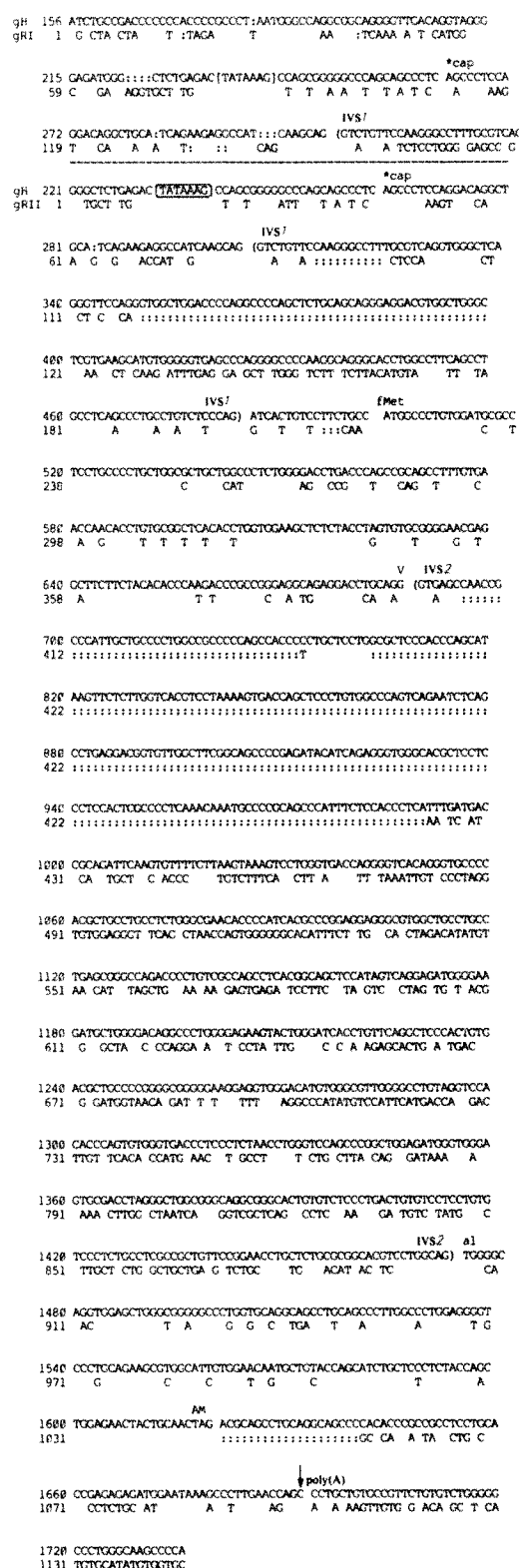
The nucleotide sequence in one reading frame predicts the amino acid sequence of human preproinsulin. It also establishes the presence of a 786-base pair intervening sequence within the DNA region encoding the C-peptide of proinsulin (IVS2). There is some ambiguity in the precise localisation of the intervening sequence. If one applies the GT/AG rule of Breathnach *et al.*²⁵ (GT at the 5' terminus, and AG at the 3' terminus of an intervening sequence), the intervening sequence occurs between the first and second nucleotides of the GUG which encodes Val 39 of proinsulin (Fig. 3). The rat insulin II gene possesses a shorter intervening sequence (499 base pairs) in exactly the same position²⁰; the rat insulin I gene lacks this intervening sequence^{19,20}. Besides the difference in length, there are other notable differences between the human and rat II intervening sequence (IVS2). In the human gene, the repeated boundary sequence is a trinucleotide, GTG, whereas in the rat II gene it is a dinucleotide, AG. There are three possible splicing points for the removal of the intervening sequence in the rat insulin II gene; there are only two possible splice points in the human gene. Except for the nucleotide sequence at the junctions, there is no extended homology between the intervening sequences of the human and rat II insulin genes (see below and Fig. 4).

The 416-base pair cloned cDNA of human insulin mRNA extends from 10 bases before the start of translation to the

poly(A) tract²¹. With the exception of the 786-base pair intervening sequence described above and two positions in the 3'-untranslated region of the gene, discussed below, the sequence of this gene is identical to the cDNA clone.

The structure of the 5'-untranslated region of human insulin mRNA could not be determined from the human insulin cDNA clone since the clone lacks most of this region. However, a comparison of the DNA sequence preceding the human insulin gene with the same region in the rat insulin I and II genes should define the 5'-end of human insulin mRNA, as well as localising any intervening sequences in this region. The results of such a homology study between the rat and human genes (Fig. 4) reveal

Fig. 4 Homology of the human insulin gene with the rat insulin I and II genes. The human insulin gene sequence (gH) (Fig. 3) is compared with the sequence of the rat genes for insulin I (gRI)¹⁹ and insulin II (gRII)²⁰. 'Maximum' homology was achieved by suitable additions, indicated in the sequence by colons, and by using a computer homology program. The program allows editing and moving of sequences while comparing one against the other. The detection of important homologies is facilitated with the Maxalig subprogram which finds the 15 best alignments between the two sequences being compared, showing the homology score for each, the size of the largest common cluster (contiguous sequence of matches) and the location of such a cluster. Another feature enables the user to select subsequences and work with these independently of the parent sequences. This may be carried out to any desired level to set subsequences of subsequences, and so on. This was used to obtain maximum homology by comparison of similar functional regions, for example, IVS1 of human and rat. The upper, continuous sequence is that of the human gene. The lower, discontinuous sequence indicates the nature of the differences between the human and rat genes. The absence of a nucleotide in the lower sequence indicates that the human and rat sequences are the same; the presence of a colon in either sequence indicates a gap equal in length to the number of colons to be added to achieve maximum homology. The Hogness sequence, TATAAAG, is boxed. The positions of the putative 5' end (capping site; *cap) and 3' end (poly(A) addition site; arrow, poly(A)) of the mRNA are indicated. The intervening sequences are indicated within parentheses and the left and right borders marked IVS1 or 2, respectively. The occurrence of IVS2 between the first and second base of the valine codon at proinsulin amino acid position 39 is indicated by a 'V' at the left hand boundary and 'al' at the right. The initiation codon for preproinsulin is designated 'fMet'. The numbers in the left margin refer to the human sequence (numbered according to Fig. 3) and the rat I or II sequence numbered according to refs 19 or 20, respectively. The upper panel presents the homology of the human insulin gene with the rat insulin I gene in the region before the first intervening sequence (IVS1), and the lower panel the entire homology of the human insulin gene with that of rat insulin II.



three regions of sequence homology in the 5' portion of the gene. The human and rat I genes (the rat II gene sequence does not extend this far²⁰) are homologous around nucleotide 186 of the human sequence (numbers are derived from the sequence in Fig. 3) and preceding a presumptive transcriptional initiation site. The second region (nucleotides 231–310) is centred around the 'Hogness sequence' TATAAAG (positions 233–239); no other TATAAAG-like sequence occurs in the 1,789 base pairs which have been sequenced. In the rat insulin genes, this region of homology includes the 5'-end (capping site) of the mRNA, the 43 bases corresponding to the 5'-untranslated portion of the

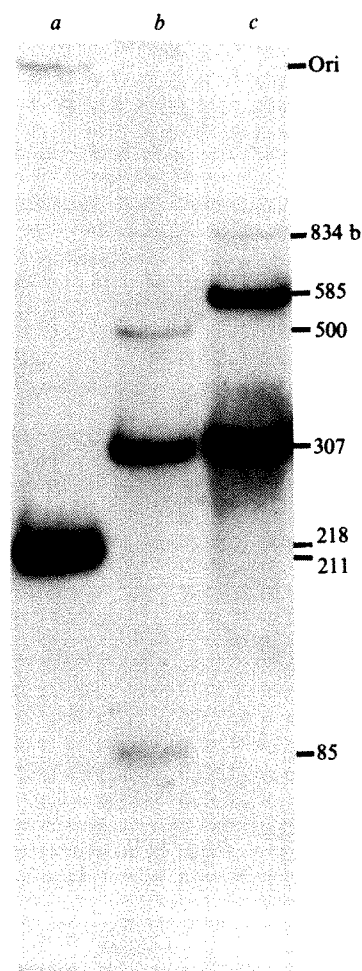


Fig. 5 Comparison of *Pst*I digestions of human insulin gene isolates gHI 12.5 and gHI 14.1. After *Pst*I digestion the DNA fragments were separated by electrophoresis in reversibly cross-linked 6% acrylamide–1% agarose gels²⁸. The fragments were transferred to nitrocellulose and hybridised with labelled human insulin cDNA. The size of hybridising fragments in base pairs are indicated. 'Ori' (origin) is the top of the gel. The lanes are *Pst*I digestions of *a*, human insulin cDNA clone, pCHI-1²¹; *b*, human insulin genome clone, gHI 12.5; *c*, human insulin genome clone, gHI 14.1. From the sequence (Fig. 3) and restriction mapping the order of *Pst*I fragments in lane *b* is (in base pairs) 5'–307, 834 (27) 85, 500–3'. The 27-base pair fragment is not seen as it migrated too rapidly.

mRNA as well as the 5'-boundary of intervening sequence 1 (IVS1). The extensive homology present in this segment (Fig. 4) suggests it contains the cognate region of the human insulin gene. Therefore, we tentatively assign the A at position 263 (Fig. 3) as the 5'-end of the mRNA. This is 24 base pairs after the end of the TATAAAG sequence. The start of the intervening

sequence is 43 base pairs after this A residue in the rat II gene. Taking into account the homology and applying the GT/AG rule, this segment of the human insulin gene is only 42 base pairs long. It therefore appears that a similar intervening sequence occurs in the human insulin gene with its boundary at guanosine 305 (G-305). The third region of homology in this section of the gene is centred at nucleotide 483, 17 base pairs before the ATG which encodes the fMet of preproinsulin. This undoubtedly corresponds to the 3'-boundary of the intervening sequence which began at G-305. Thus, the human insulin gene contains an intervening sequence of 179 base pairs in the DNA segment encoding the 5'-untranslated region of insulin mRNA; this is 60 base pairs longer than the 119-base pair intervening sequence in the rat insulin genes. In the human gene the 3'-border of this intervening sequence is 3 base pairs further from the fMet encoding ATG as compared to the corresponding sequence in the rat genes.

Thus the 5'-untranslated region of human insulin mRNA is 59 nucleotides; in rat insulin mRNA, it is 57. Both human and rat insulin genes contain an intervening sequence of different length in the DNA which encodes this portion of the mRNA, although the exact position from the boundaries of the leader segment are slightly different. The sequence reiterated at the boundaries is different between the two species as is the number of splicing frames (human: 4; rat: 5). As observed for IVS2 in the C-peptide region there is no obvious homology between this intervening DNA segment (IVS1) in the human and rat genes except at the boundaries.

In contrast to the substantial nucleotide sequence homology between the rat and human insulin genes evident in the 5' flanking region, the coding regions, and the 21 bases at the end of the 3'-untranslated region before the poly(A) site, there is no homology in the region extending further in the 3' direction.

Finally, the 1,789-base pair segment which contains the gene and adjacent regions is very GC-rich. The mean molar GC content of human DNA is 38.0% (ref. 26), whereas this segment has a content of 64.8%. The high GC content is uniformly present in all regions sequenced. The rat insulin genes also have a GC composition of 55%, which is greater than rat DNA, 40% (ref. 27).

Allelic variation

Restriction endonuclease analysis of the two human insulin gene isolates demonstrated a *Pst*I site in gHI 12.5 (Fig. 5, lane *b*) which is absent in gHI 14.1 (Fig. 5, lane *c*). Clone gHI 14.1 possesses a 585-base pair fragment instead of the 500- and 85-base pair fragments of gHI 12.5. The nucleotide sequence of the insulin gene of gHI 12.5 indicates that this additional *Pst*I site is centred at nucleotide 1,628 in the portion of the gene encoding the 3'-untranslated region of the mRNA. There are also two differences between the sequence of the human insulin cDNA clone, cHI-1 (ref. 21), and the genomic clone, gHI 12.5 in this region (Fig. 6); a C to T transition at nucleotide 1,628 which results in a *Pst*I site in gHI 12.5, and a C to A transversion at nucleotide 1,641. It is difficult to exclude the possibility that the differences observed in the cloned cDNA did not occur during cDNA synthesis. Nevertheless, the similarity in the restriction maps of the two genome isolates outside the insulin gene region (Fig. 1a) suggests that the observed differences reflect allelic variation. Furthermore, this interpretation indicates that the individual from whom the DNA was obtained for cloning was heterozygous since insulin sequencing as well as genetic studies²⁹ suggest only a single insulin gene per haploid genome complement.

Structural features

Present models of eukaryotic transcription propose that the primary transcript (pre-mRNA), which is co-linear with the DNA, is capped and polyadenylated before removal of any intervening sequences^{30,31}. Messenger RNA biosynthesis can be

regulated at any of these steps. If the signals for these processes are dependent upon the primary structure of the DNA or RNA, the features common to both the human and rat insulin genes may indicate regulatory regions conserved during evolution.

As indicated in Fig. 4, there are two regions of sequence homology in the DNA segment preceding the sequence encoding the 5'-terminus of the mRNA. One region is around the sequence TATAAAG (G239), 24 base pairs before the start of transcription. This sequence is thought to interact with RNA polymerase to facilitate initiation of transcription³⁰. The other region is centred at A 184, 77 base pairs in front of the capping site. In the human but not the rat insulin gene this region is also part of a quite large imperfect palindrome (positions 155–213). A similar palindrome but of different sequence is present in mouse α -globin³² but not in the mouse β -globin genes^{33,34}. Since structures of this type in bacterial genes are often involved in the interaction with regulatory proteins³⁵, the human gene palindrome may also be a binding site for an insulin-specific regulatory protein.

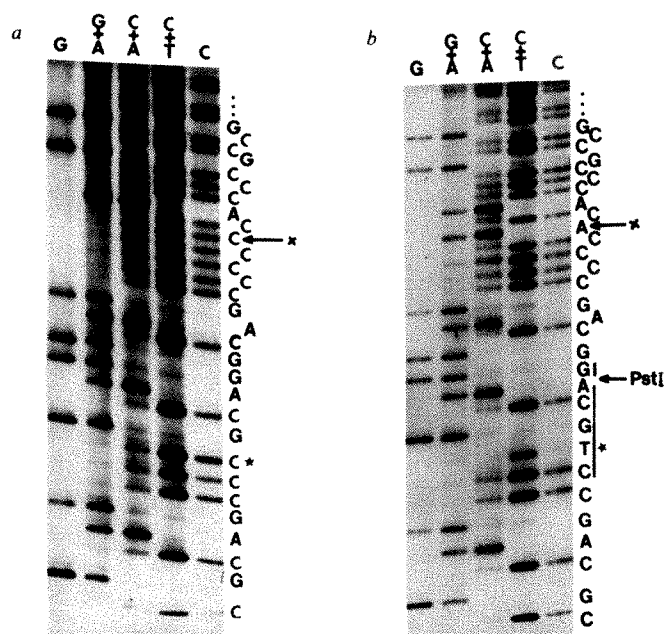


Fig. 6 Comparison of the sequence of the DNA region encoding the 3'-untranslated portion of insulin mRNA of gHI 12.5 (a) and cHI 1 (b). The sequence differences are indicated. The single *PvuII* site within the gene (gHI 12.5) or cDNA (cHI 1)²¹ was labelled with [γ -³²P]ATP and polynucleotide kinase. After secondary restriction enzyme digestion, the 5'-end-labelled fragments were sequenced by the procedure of Maxam and Gilbert²⁴. The chemical cleavage products were separated by electrophoresis in a 0.38-mm thick 15% polyacrylamide gel.

There are a number of direct repeat sequences located in the region immediately preceding the TATAAAG sequence. The sequences GGAAATT (positions 119–124 and 131–136), CC(G or C)ACCCC (positions 162–169 and 170–177), and GG(T or C)AGGGG (positions 195–202 and 209–216) each occur twice while the sequence CAGCC (positions 139–143, 145–149, and 152–156) occurs three times. Also, the sequence at the 5'-end of the mRNA, AGCCCTC (positions 263–269), is repeated immediately upstream at positions 256–262. The 5'-terminus of the silk fibroin mRNA is also repeated in a similar manner³⁶. Since most of these features are only characteristic of the human insulin gene and are not observed in the rat insulin genes, their physiological significance is not known. However,

since there are two insulin genes in rat and only one in human, some aspects of control may be different.

There is no obvious homology between the human and rat genes in the DNA sequence to the 3' side of the common 21-base pair sequence surrounding the AAUAAA sequence at the end of the insulin mRNA. Hence, if there are regulatory signals which occur 3' distal to this point, they are not a common feature of the insulin genes.

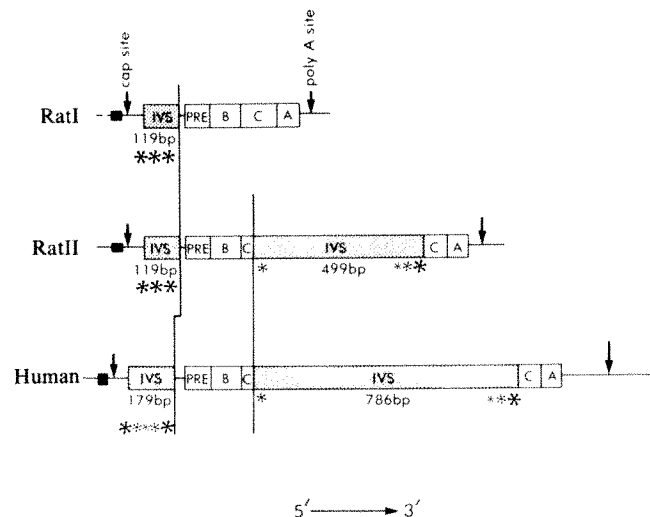


Fig. 7 Schematic comparison of human and rat insulin genes. The topology of the two rat insulin genes (I and II) and the single human insulin gene is displayed. The coding sequences for the peptide chains (pre-, B, C and A) of preproinsulin are represented by the clear boxes. Intervening sequences (IVS) are distinguished by the shaded areas with the length of each intervening sequence indicated below. The extent and position of nucleotide homology between intervening sequences of the two species is represented by the size of the asterisks—the larger the asterisk the greater the homology. Vertical lines describe the positions at which intervening sequences occur. The internal intervening sequence in the rat II and the human gene occurs in exactly the same position (valine 39 of the C-peptide region) whereas the position of the intervening sequence located in the 5'-untranslated region is displaced by three nucleotides in the human gene as compared with both rat genes. Also indicated are the sites for polyadenylation and capping (shown by arrows) as well as the Hogness box, a potential site for the initiation of transcription (indicated by the small black box). The arrow indicates the direction of transcription.

The signals in the nucleotide sequence which direct the capping and intervening sequence excision reactions are not obvious. However, as observed for other mRNAs²⁰, the 5'-untranslated region of the human insulin mRNA does possess some elements of secondary structure, whose possible regulatory role, if any, is undetermined. From a comparison of the similar regions of other genes, both the intervening sequences in the human insulin gene conform with the GT/AG rule proposed by Breathnach *et al.*²⁵. Also, as there are several ways in which each intervening sequence can be removed and still preserve the sequence of the mRNA (see ref. 37 for review), the structure(s) of the pre-mRNA molecule which affect intervening sequence removal remain elusive. The conservation of sequence homology around the boundaries of intervening sequences of the rat and human insulin genes suggest that the sequences of this region might be important. This homology is most extensive on the 5' side of the splice point, that is, before the GT at the 5' and the AG at the 3' borders of the intervening sequences. However, these features are not evident in comparisons between the rabbit and mouse β -globin genes³⁸.

Discussion

There now appear to be at least two types of mammalian insulin gene. The genes for human and rat II have two intervening sequences, while rat I has a single intervening sequence (Fig. 7). All three contain an intervening sequence in the DNA which encodes the 5'-untranslated or leader position of insulin mRNA. The position of this intervening sequence is the same in the rat I and II genes, but is displaced by 3 base pairs in the human gene. The human and rat II insulin genes contain an additional intervening sequence at exactly the same position in the C-peptide encoding region of the gene. The similarity in the structure of the human and the rat II insulin genes suggests that the ancestral mammalian insulin gene will contain two intervening sequences. It has been assumed that the human haploid genome contains a single insulin gene since only one insulin protein has been isolated. The two independent isolates of the human insulin gene described here have identical restriction endonuclease maps (Fig. 1a) in the region outside the gene. This is not usually the case for non-allelic genes, for example the rat I and II insulin genes^{19,20}. The two human insulin gene isolates, however, differ by at least one base change which results in the elimination of a *Pst*I site in the region corresponding to the 3'-untranslated portion of the mRNA. These data indicate that the clones represent alleles of a single insulin gene. Data from experiments on a mutant form of insulin which results in diabetes²⁹ as well as studies on familial hyperproinsulinaemia³⁹ are consistent with this hypothesis and furthermore indicate co-dominant expression of the two alleles of a diploid heterozygote. In rats and presumably in other species with two insulin genes, the single gene was duplicated and at least in rats has lost one of the intervening sequences. The mechanism of elimination of the intervening sequence from the rat I gene must have been precise since the amino acid sequence and nucleotide sequence around the splice have been conserved. Although the gene sequences which encode human and rat insulin mRNA and several sequences before the gene are highly conserved, the internal regions of the intervening sequences of these genes are highly divergent. In fact, so much so that it is difficult by homology studies to discern any meaningful regions of homology within the internal regions of the intervening sequence and therefore impossible to deduce anything about the evolution of these portions of the insulin gene or the cause of their different sizes.

Evidence from viral systems suggests that at least one intervening sequence in the mRNA precursor is necessary for normal mRNA accumulation⁴⁰. This would indicate a requirement for one intervening sequence in the insulin gene. The role of additional intervening sequences in insulin and other genes is unknown. In the case of rat insulins the additional sequence does

not substantially affect biosynthesis since both rat insulins are present in approximately equal proportions^{41,42}. It is interesting, however, that it is present in that region of the gene which encodes the most variable portion of the molecule (the C-peptide), yet Gln and Val adjacent to the splice have been highly conserved. Moreover, the region around the splice has been deleted in the insulin related proteins, insulin growth factors I and II and relaxin¹⁷. An examination of the genes for these proteins as well as the insulin genes of lower vertebrates might give us insight into the structure of the primordial gene.

The causes of diabetes are still poorly understood. The disease can be classified into insulin-dependent and -independent forms. The insulin-dependent form of the disease may result from a destruction of the insulin-producing β cells by chemicals or viral infection^{8,9}. However at least in one instance this form of the disease is the consequence of a mutated insulin²⁹. Its study by Tager *et al.*²⁹ established a genetic basis for this form of the disease in some individuals and, moreover, that both human insulin alleles are expressed. In the other, more common form of the disease, insulin-independent or maturity-onset, there is a pronounced correlation between family history and diabetes^{1,7}. Thus there can be a genetic component associated with both forms of the disease. The allelic differences observed in the 3'-untranslated region of human insulin mRNA are a form of polymorphism which is not manifested in the structure of the protein but could affect insulin biosynthesis. This form of allelism might be especially relevant if it occurred in functionally important regions of the gene or mRNA, for example, the DNA region around that encoding the 5'-end of the mRNA. Presumably in normal metabolic conditions, insulin biosynthesis might be unaffected, however under metabolic stress there could be a profound effect on insulin synthesis which could result in diabetes. This hypothesis can be tested by examining the insulin genes of diabetic individuals to determine if they possess alterations. Moreover the extensive nucleotide sequence conservation between human and rat insulin genes in regions outside those encoding preproinsulin immediately suggests those regions which should be examined for their possible role in the regulation of insulin biosynthesis. These analyses as well as surrogate genetic techniques, in which normal or *in vitro* mutated insulin genes are introduced into cultured cells or *Xenopus* oocytes, should increase our understanding of the genetic factors which regulate insulin biosynthesis.

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Neural regulation of pancreatic hormone secretion by the C-terminal tetrapeptide of CCK

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In pancreatic islets a peptide corresponding to the C-terminal tetrapeptide amide of cholecystokinin and gastrin, Trp-Met-Asp-Phe-NH₂, is present in nerve terminals. This tetrapeptide amide is uniquely potent as a releaser of insulin and the other islet hormones, whereas larger cholecystokinins and gastrins as well as tetrapeptide analogues are considerably less potent. We suggest that neural release of the tetrapeptide amide is implicated in regulation of pancreatic hormone secretion.

HORMONAL peptides may also serve important roles as neuroregulators. The related gut hormones, gastrin and cholecystokinin (CCK), belong to this group of peptides. They are located in central¹⁻⁶ and peripheral nerves^{6,7}, synthesised in neuronal tissue⁸, and concentrated in synaptic vesicles from which they are released like other neurotransmitters⁹.

Recently we studied the distribution and molecular nature of CCK in peripheral nerves⁶, and in continuation of these studies we have observed CCK nerve terminals in pancreatic islets. The predominant form of CCK in these nerves seems to be the C-terminal tetrapeptide amide, Trp-Met-Asp-Phe-NH₂, common to CCK and gastrin, hereafter referred to as CCK-4. We have also examined the effect of various cholecystokinins and gastrins on the isolated, perfused pancreas and observed that CCK-4 was considerably more effective in releasing insulin than larger molecular forms of gastrin and CCK, confirming our earlier impressions in man¹⁰. The results suggest that the secretion of insulin and other islet hormones is under neural control by the tetrapeptide amide.

Structure of CCK and the application of antisera specific for different CCK sequences

CCK is closely related to gastrin both structurally and functionally. The hormones probably share a common evolutionary origin⁴. Their identical C-terminal sequence (Fig. 1) is responsible for their biological activity, and the remaining parts of the molecules modify their potency on different target tissues. Both hormones are synthesised and released as a range of molecules of different sizes and biological potencies^{2,11-16}. Because of this molecular heterogeneity and as the common sequence appears highly immunogenic, characterisation of CCK immunoreactivity by conventional radioimmunoassays and conventional immunocytochemistry is insufficient. We have, therefore, developed a range of antisera specific for different sequences of CCK-33 and gastrin-17 (Fig. 1); details of their production and characterisation have been described previously¹⁷⁻¹⁹. These antisera have proved valuable for the characterisation of CCK and gastrin cells^{4,19,20} and in identifying the molecular nature of gastrin and CCK in extracts of nervous tissue^{1,2} and in cerebrospinal fluid²¹. In the present study we have used the antisera

to examine the localisation and molecular nature of the pancreatic CCK/gastrin-like peptides. Moreover, we have studied the activity of different molecular forms of CCK and gastrin on pancreatic secretion.

Structure-activity relationship of the cholecystokinins and the islet-cell secretion

The effect of different molecular forms of CCK on the release of insulin and the other islet hormones was studied by perfusion of the isolated, porcine pancreas. The concentration of insulin, glucagon, somatostatin and pancreatic polypeptide in the effluent was determined by sensitive and specific radioimmunoassays, whose development and character have been described in detail previously²²⁻²⁵. We chose the porcine pancreas to study the effect of gut hormonal peptides²⁶, because the pig is the only species for which the structure of most gut hormones, including CCK, is known. The perfusion technique and the reliability of this *in vitro* model has been reported in detail previously^{26,27}.

The cholecystokinins stimulated the secretion of all pancreatic hormones in a dose-related manner (Fig. 2). CCK-4 was by far the most potent peptide. Even at the lowest concentration (10^{-10} M), CCK-4 was a powerful secretagogue. The intact tetrapeptide amide with free Trp- and -Phe-NH₂ termini seemed necessary for the activity. Thus, synthetic peptides with NH₂-terminal modifications, such as removal of tryptophan (CCK-3), extension by glycine (CCK-5, gastrin pentapeptide) or by BOC- β -alanine (Peptavlon) greatly reduced the secretagogue activity. C-terminal modifications also reduced the potency. Thus, the deamidated tetrapeptide, Trp-Met-Asp-Phe-OH, stimulated insulin secretion only at the highest concentration tested (10^{-6} M), and it was without effect on glucagon, somatostatin and pancreatic polypeptide secretion (Fig. 3).

The larger molecular forms, CCK-8 and CCK-33, were significantly less potent stimulators of the endocrine pancreas (Fig. 2). As the tetrapeptide amide constitutes the active site also of the gastrins, we compared the effect of CCK-4 with the

principal molecular form of the gastrin, the heptadecapeptide amide gastrin-17 which, likewise, was several-fold less potent than CCK-4 (Fig. 2). In contrast to their weak effects on the endocrine pancreas, the larger hormonal peptides (CCK-8, CCK-33 and gastrin-17) were all more effective than CCK-4 in stimulating exocrine, pancreatic secretion (Table 1). This observation is consistent with the absence of innervation of the exocrine pancreas by CCK nerves (Figs 4 and 5).

Innervation of the pancreatic islets by cholecystokinin nerves

The immunocytochemical studies were conducted on pancreatic tissues from pig, cat and hamster. Antisera specific for the C-terminus common to CCK and gastrin revealed immunoreactive nerves occurring in two distinct locations: islets and ganglia (Figs 4 and 5). These nerves enter the pancreas in large trunks which also contain numerous non-immunoreactive fibres. In contrast to the smooth fibres observed in nerve trunks, those occurring in and around ganglia and islets had a beaded appearance, suggestive of axon terminals with varicosities. The widespread distribution of immunoreactive nerves within the pancreatic islets and ganglia suggests a true innervation of the endocrine cell populations. The failure to detect immunoreactive nerve cell bodies in the pancreas, as well as the strong innervation of the ganglionic cells, suggests an extrinsic source for the nerves.

Our attempts to demonstrate the nerves with C-terminal-specific antisera were guided by previous observations on brain tissue showing that the CCK nerves contained a preponderance of a C-terminal tetrapeptide-like component^{2,6,28} which was detected only by these antisera⁶. The immunocytochemical results were corroborated by absorption controls showing that only peptides containing the C-terminal tetrapeptide amide sequence were able to inhibit the staining reaction (compare Fig.

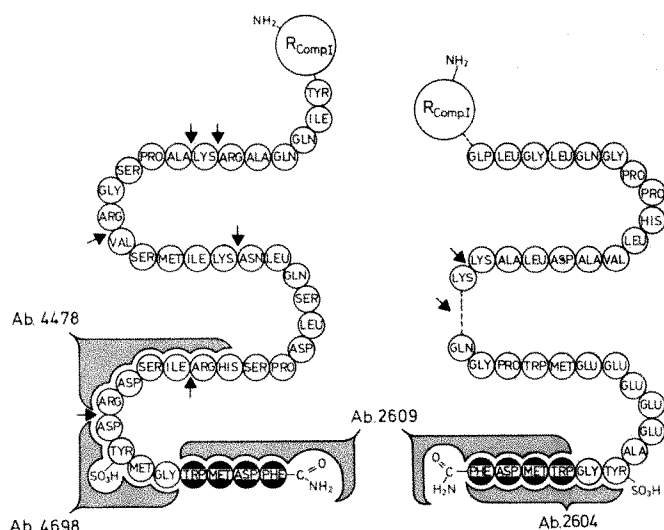


Fig. 1 The amino acid sequences of porcine cholecystokinin (left) and gastrin (right). The largest molecular form of CCK with known structure is a nonatriacontapeptide amide (CCK-39)^{36,37}. Molecular forms larger than CCK-39 have been found in nervous tissue and intestinal mucosa^{1-3,11}. These forms² have an additional extension at the NH₂-terminus of CCK-39. The amino acid sequence of the NH₂-terminal extension (R_{comp.1}) is not yet known. The largest molecular form of gastrin with known structure is a tetratriacontapeptide amide (gastrin-34)³⁸. A molecular form larger than gastrin-34, component I, has been found in serum¹⁴, gastrointestinal mucosa³⁹, the pituitary¹ and the vagal nerve⁷. Gastrin component I corresponds to gastrin-34 with an NH₂-terminal extension. The amino acid sequence of this extension, (R_{comp.1}) is not yet known. The shaded amino acid residues indicate the common C-terminal tetrapeptide amide sequence of CCK and gastrin. The arrows indicate points of tryptic cleavage. The brackets indicate the specificities of the antisera. Production and characterisation of these antisera have been described previously^{2,18}.

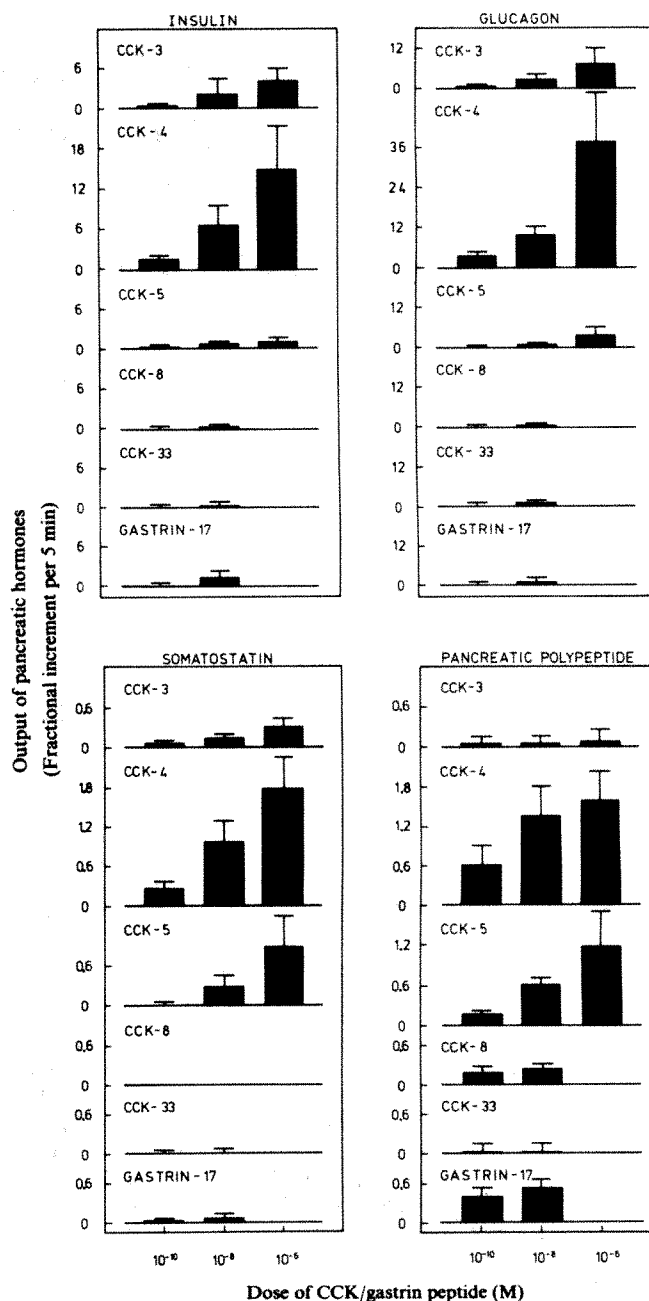


Fig. 2 Secretion of insulin, glucagon, somatostatin and pancreatic polypeptide from the isolated perfused, porcine pancreas in response to 5-minute stimulations with CCK and gastrin peptides. The CCK/gastrin peptides were administered in concentrations of 10^{-10} , 10^{-8} and 10^{-6} M. The mean (\pm s.e.m.) incremental hormone output (\sim fold increase) above the output in the corresponding 5-min prestimulatory period was calculated. Each dose of each peptide was administered in seven perfusion experiments, except CCK-4, which was given during 11 perfusion experiments. CCK-3, -4, -5 and -8 correspond to the COOH-terminal tri-, tetra-, penta- and octapeptide amide of CCK-33. The preparation and technique for the perfusion experiments have been described in detail previously^{25,26}. In the present experiments the glucose concentration in the perfusate was 5–7 mM. The response of the four pancreatic hormones to stimulation with gastrin and CCK peptides showed only little dependency of the glucose concentration in the perfusion medium within the 5–7 mmolar range. Studies of the effect of other large molecular forms of gastrin and CCK (gastrin-34, gastrin-14 and CCK-39) will be reported elsewhere (Jensen, S.L. *et al.*, in preparation).

4). Thus, a system of pancreatic nerves, storing either the free tetrapeptide amide or peptides containing this sequence, appear to innervate both the islet parenchyma and ganglia.

Attempts to localise the immunoreactive material at the ultrastructural level were unsuccessful. Embedding of formaldehyde-fixed tissue in resins or paraffin abolished immunoreactivity. Hence, post-embedding staining²⁹ was not possible.

Table 1 Exocrine secretion (ml h⁻¹, mean and range) from the isolated perfused porcine pancreas during stimulation with CCK/gastrin peptides

	Preperiod	10 ⁻¹⁰ M	Preperiod	10 ⁻⁸ M	n
CCK-4	0.15 (0–1.1)	0.1 (0–0.9)	0.1 (0–0.48)	1.2 (0.48–2.28)	11
CCK-8	0.1 (0.1–1.2)	1.5 (0.96–3.60)	0.2 (0.1–0.4)	6.0 (3.0–6.0)	7
CCK-33	0.1 (0–0.3)	2.2 (0.1–3.3)	0.6 (0–0.6)	6.0 (2.4–9.0)	8
Gastrin-17 (s)*	0.3 (0.2–0.9)	2.4 (1.0–4.8)	0.4 (0.1–1.3)	10.8 (5.0–14.1)	10

* (s), sulphated variant.

Instead we stained cryostat sections, which subsequently were embedded in Epon-812. Ultrathin sections revealed nerve terminals containing stained vesicles of varying diameters. The significance of this result is doubtful in view of the difficulties in carrying out appropriate controls on equivalent sections.

Apart from the nerves described above the pancreas also contains scattered endocrine cells displaying C-terminal gastrin-CCK immunoreactivity. Such cells were numerous in the feline

pancreas, scarce in the hamster pancreas and absent from the pig pancreas. As shown in a previous study, these immunoreactive cells also react with gastrin-specific, but not with CCK-specific antisera, and they are distinct from islet D (somatostatin) cells³⁰. In the feline pancreas, these cells occur in both ducts and islets, and are also scattered in the exocrine parenchyma. In the hamster, rare immunoreactive cells were confined to the ducts and exocrine parenchyma. Recent double staining experiments

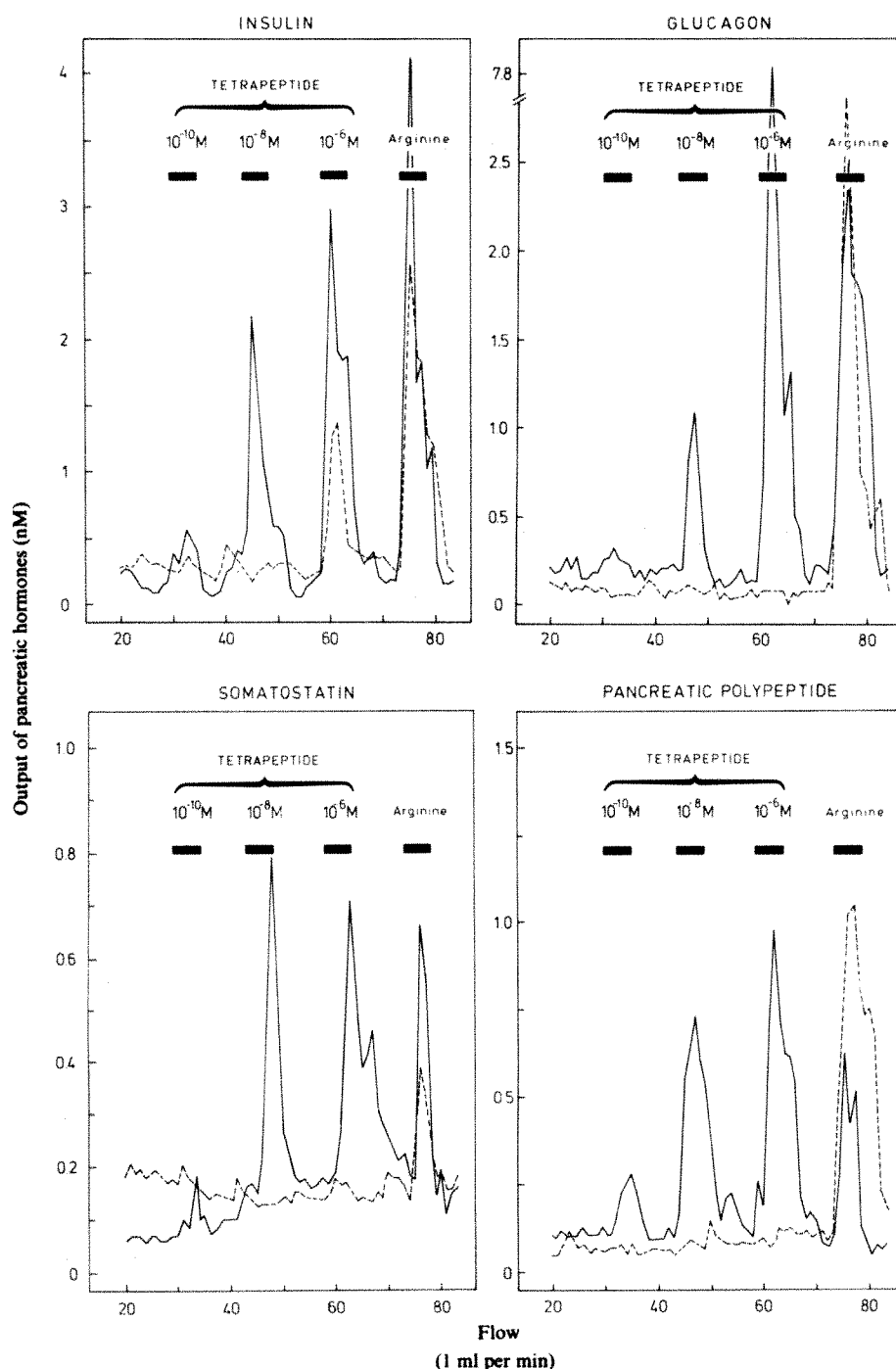


Fig. 3 Secretion of insulin, glucagon, somatostatin and pancreatic polypeptide from the isolated, perfused porcine pancreas in response to 5-min stimulations with the intact tetrapeptide amide (Trp-Met-Asp-Phe-NH₂; solid line) and with the deamidated tetrapeptide (Trp-Met-Asp-Phe-OH; broken line). Each line represents a typical perfusion experiment in which the tetrapeptide was administered in the three doses indicated. CCK-4 was given in 11 experiments (for mean output and s.e.m., see Fig. 2), and the deamidated tetrapeptide in four experiments, all with results as shown above. Each perfusion experiment was terminated by a 5-min stimulation with L-arginine (5 mM) to control optimal function of the preparation. For further details about technique, see refs 26 and 27.

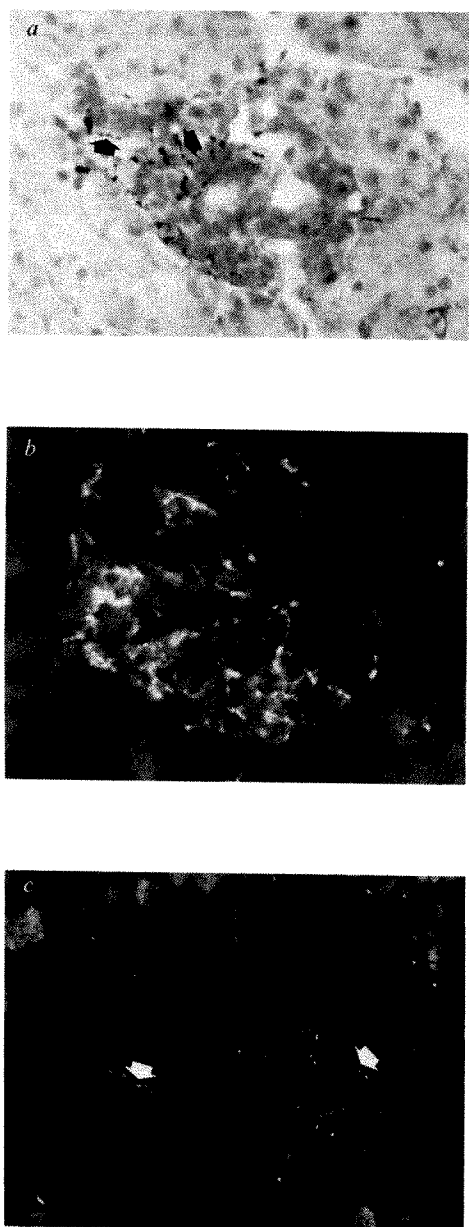


Fig. 4 Sections of cat pancreatic islets stained with antiserum 4562, recognising the common C-terminal tetrapeptide amide of cholecystokinin and gastrin, Trp-Met-Asp-Phe-NH₂. In *a*, the site of antigen-antibody reaction has been revealed with the peroxidase-antiperoxidase (PAP) method of Sternberger^{6,29}, whereas in *b* indirect immunofluorescence has been used ($\times 295$). Note the occurrence of numerous varicose immunoreactive nerve terminals, which surround the islet cells in an innervation-like pattern (arrows). Adult, freely fed cats ($n = 10$), hamsters ($n = 10$) and pigs ($n = 1$) were studied. The pig pancreas was perfused in situ with a 4% paraformaldehyde solution in 0.1 M sodium phosphate buffer pH 7.3, following cessation of stimulation experiments. Pancreatic slices were dissected out, postfixed in the same fixative for 1–2 h and rinsed in 20% sucrose in 0.1 M sodium phosphate buffer pH 7.3, overnight. Cats and hamsters were anaesthetised (mebumal/chloralose) and perfused via the heart with the formaldehyde solution. Following postfixation and rinsing in sucrose all pancreatic specimens were frozen in melting Freon 22 and sectioned in a cryostat. Immunocytochemical staining was carried out with antisera as described elsewhere⁶. The site of antigen-antibody reaction was revealed by immunofluorescence or by the peroxidase-antiperoxidase (PAP) procedure^{6,29}. Controls included conventional staining controls²⁹ as well as absorption controls including pretreatment of the antisera for 24 h at 4 °C with 100 μ g per ml diluted antiserum of *a*, pentagastrin (ICI); *b*, synthetic human gastrin I (ICI); *c*, synthetic caerulein (Farmatolia); and *d*, 99% pure porcine CCK-33 (V. Mutt). Absorptions against these peptides, but not against peptides lacking the common COOH-terminal tetrapeptide sequence (vasoactive intestinal polypeptide, substance P, ACTH, somatostatin) abolished staining. *c*, Section of hamster islet stained by indirect immunofluorescence as in *b*. Weak unspecific fluorescence (autofluorescence) is noted in the zymogen cells at the periphery of the islets. Unlike the fluorescence of the islet nerves, this fluorescence remains in control sections.

(for technical details, see ref. 31) in cat pancreas have now shown that the CCK nerves are primarily associated with glucagon cells in the islets.

The molecular nature of cholecystokinin in the pancreas

We extracted pancreatic specimens only from adult mammals in which we had ascertained that the immunoreactivity was located exclusively or almost exclusively in nerves, that is, hamsters and hogs. Porcine pancreatic tissue was obtained from a local abattoir 20 min post mortem. Hamster pancreas was dissected from anaesthetised animals. The tissue was immediately frozen at -70°C until extraction. Samples of frozen tissue weighing 0.5 to 2 g were immersed in boiling water (0.1 g ml^{-1} , pH 6.6), boiled for 20 min, homogenised and centrifuged (2,000g, 20 min). After decanting the first supernatant the pellet was extracted with 0.5 M acetic acid for a further 15 min at 4 °C, followed by homogenisation and centrifugation (2,000g, 20 min). The water and acetic acid supernatants were applied to calibrated Sephadex columns (Fig. 6).

The chromatography, monitored by the different radioimmunoassays, disclosed the presence of five different molecular forms of CCK in the porcine pancreas (Fig. 6). The component with the largest molecular weight eluted in a position before CCK-39, and immediately after the void volume. The next three components eluted in positions corresponding to those of CCK-33, the C-terminal dodecapeptide of CCK (CCK-12), and the C-terminal octapeptide of CCK (CCK-8). Finally, a fifth component eluted at a position corresponding to the C-terminal tetrapeptide amide (CCK-4). When synthetic CCK-4 was used as standard for measurement of the fifth component, this component was seen to be far more abundant than the remaining CCK components (Fig. 6c). Apart from the immunoreactivity corresponding to CCK-4, specific gastrin immunoreactivity was not found in the porcine extracts. The nature of the different CCK-components was corroborated by re-chromatography on Sephadex G-50 and G-25 columns and by chromatography with a urea gradient. Using appropriate standards (CCK-33 for components I and II; CCK-8 for III and IV; and CCK-4 for V) the following concentrations of each component (I–V) were found: 1.2 ± 0.5 , 0.3 ± 0.1 , 1.5 ± 0.7 , 3.2 ± 1.1 and 19.6 ± 7.1 pmol per g porcine pancreas (mean \pm s.e.m., $n = 4$). Extracts of pancreas for hamsters contained less immunoreactivity, but again the predominant component corresponded to CCK-4. An additional component eluting in a position corresponding to heptadecapeptide gastrin was assumed to have originated from the scarce endocrine gastrin cells in the hamster pancreas.

Several lines of evidence support the contention that the fifth component described above is identical to CCK-4: (1) The

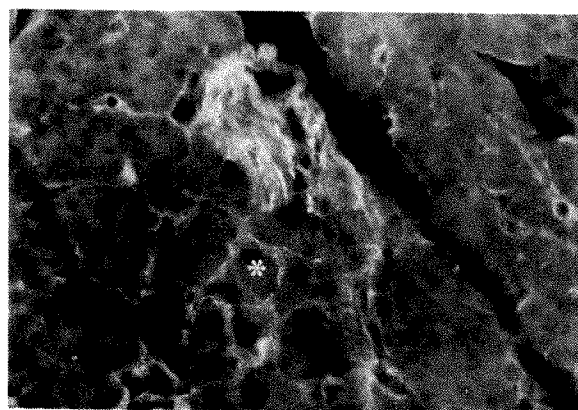


Fig. 5 Section of cat pancreas reacted with As 4562 in the indirect immunofluorescence procedure. Numerous preterminal nerves are seen to enter an intrapancreatic ganglion and to innervate non-immunoreactive ganglionic cell bodies (asterisk) ($\times 350$).

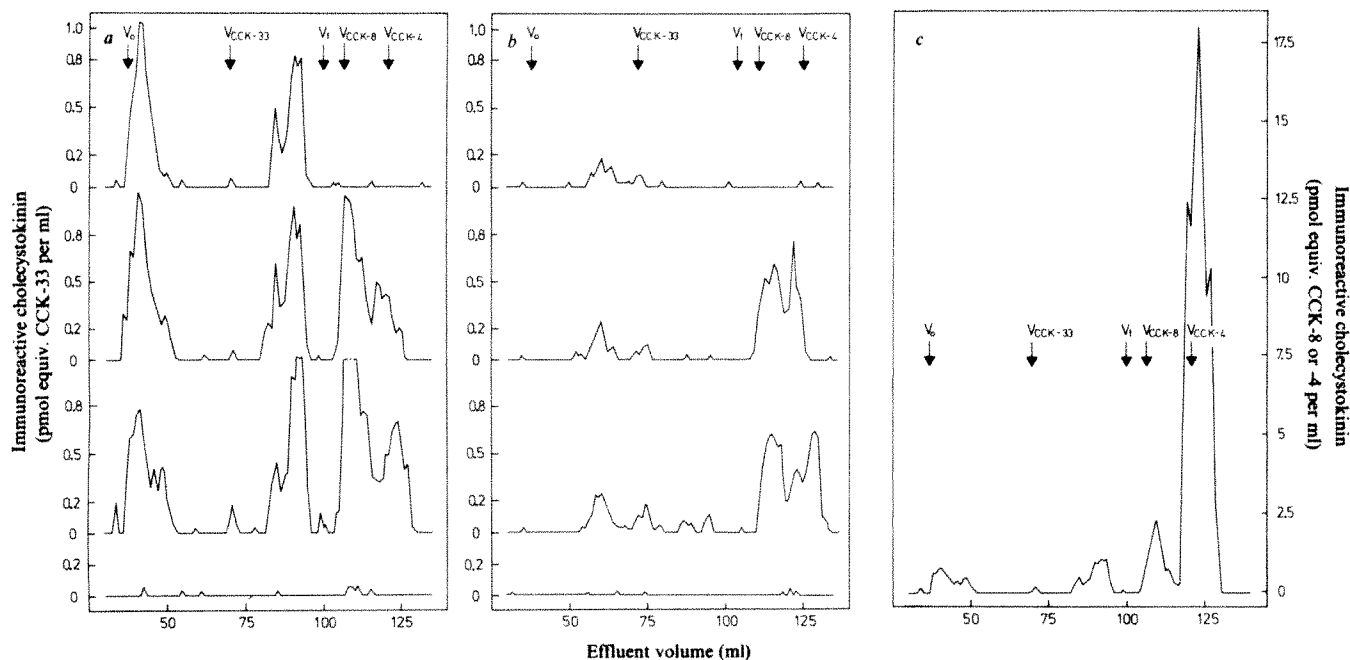


Fig. 6 Gel chromatography of extracts from the porcine pancreas, in which all cholecystokinin/gastrin immunoreactivity was confined to nerve fibres. Specimens of pancreatic tissue were obtained 20 min post mortem and immediately frozen. While frozen the tissue was cut in pieces weighing a few mg. The pieces were immersed in boiling water, for 20 min (0.1 g ml^{-1}), and subsequently homogenised for 5 min. After centrifugation, samples of the supernatant (panel *a* of the figure) were applied to calibrated Sephadex G-50 superfine columns ($10 \times 1,000 \text{ mm}$) eluted at 4°C with 0.02 M veronal buffer $\text{pH } 8.4$, at a flow rate of 4 ml per h . After decantation of the first supernatant, the pellet was extracted by 0.5 M acetic acid and rehomogenised for 5 min. After centrifugation, samples of the acid supernatant (panel *b*) was applied to columns calibrated and eluted as described above. The elutions of the boiled water extracts as well as the acetic acid extracts were monitored by four different radioimmunoassays, whose development and character has been described in detail elsewhere¹⁸. The first (upper) diagram in panel *a* and *b* illustrates the elution pattern as measured with CCK antiserum 4478, specific for sequence 20–25 of CCK-33 (see Fig. 1). The second diagram in panels *a* and *b* illustrates the elution pattern using CCK-antiserum 4698, specific for sequence 30–33 of CCK-33 (see Fig. 1). The third diagram in panels *a* and *b* illustrates the elution pattern using gastrin antiserum 2609, specific for sequence 30–33 of CCK-33 (see Fig. 1). Monoiodinated ^{125}I -CCK-33 (ref. 18) was used as tracer and porcine CCK-33 as standard for the three mentioned antisera. The fourth (lower) diagram in panels *a* and *b* illustrates the elution pattern as measured with the gastrin-specific antiserum no. 2604 (ref. 17) used for further control of the specificity. Antiserum 2604 shows a negligible cross-reactivity with CCK when monoiodinated gastrin-17 was used as tracer⁴⁰. Synthetic human gastrin-17 was used as standard with antiserum 2604. Panel *c* illustrates the Sephadex G-50 elution pattern of the boiled water extract as monitored by the assay using antiserum 2609 and ^{125}I -CCK-33. Panel *c* thus corresponds to the third diagram in panel *a*, the difference being that synthetic CCK-4 was used as standard instead of CCK-33 for measurement of the immunoreactivity eluted in the position corresponding to that of CCK-4. Note the difference in order of concentration (1:5) on the ordinates of panel *a* and panel *c*. (For further details on the immunoreactivities of different molecular forms of CCK, see refs 18 and 28.)

component was detected only with gastrin antisera which are specific for the tetrapeptide amide sequence common to gastrin and CCK (Fig. 1, 4–6). These antisera are highly sensitive to modifications of the tetrapeptide amide structure. Deamidation, removal of NH_2 -terminal tryptophyl residue, exchanges or insertions of other amino acids almost abolish the antibody binding (unpublished results). Thus, a C-terminal tripeptide amide would not be detectable. (2) The component eluted in a position corresponding to that of synthetic CCK-4 in different gel chromatographic systems. (3) The extracts contained the complementary NH_2 -terminal fragment of CCK-8, detected by antiserum 4698, which is specific for the sequence¹⁸, Asp-Tyr-(SO_3H)-Met-Gly (Fig. 1). The presence of a peptide resembling this NH_2 -terminal fragment suggests that the C-terminal peptide is produced by cleavage of CCK-8, and that it has a size corresponding to the tetrapeptide amide, as a larger C-terminal peptide, would require a smaller and hardly detectable NH_2 -terminal fragment.

The molecular forms of CCK in extracts of porcine pancreas correspond to the components in extracts of porcine nervous and intestinal tissue². The CCK-4-like component also predominates in these tissues^{28,32}. The predominance suggests that CCK-4 is the main product of the immunoreactive nerves in the pancreas. This accords with the demonstration of islet nerves with antisera specific for the C-terminus and with the weak and inconsistent staining of the islet nerves with antisera specific for other sequences of CCK-33. The larger components are presumably biosynthetic precursors in accordance with the biosynthetic relationship found for the different molecular forms of CCK in rat cerebral cortex^{8,33}.

Discussion

Our results suggest that CCK-4 (Trip-Met-Asp-Phe- NH_2) is present in CCK nerves in the pancreas and is involved in the regulation of islet cell functions. The present results, as well as those from previous studies in man¹⁰, show that CCK-4 has an immediate and potent effect on islet cell secretion.

The origin of the CCK-containing nerves in the pancreatic islets is unknown. However, CCK nerves are present in many regions of the body. Thus, we have reported that human and porcine brains contain CCK in quantities 10 to 100 times greater than those of other brain peptides^{1,2}; and that cerebral CCK is located in nerves in most regions of the central nervous system⁶. We have also found that the CCK in cerebral neurones fulfils several of the criteria for neurotransmitter function^{6,8,33}. In guinea pigs CCK is present in peripheral nerves which are particularly abundant in the muscular wall of colon⁶. We also observed a high density of CCK nerves in the coeliac-superior mesenteric ganglion⁶. The predominant molecular form of CCK in the peripheral nervous system corresponded mainly to CCK-4 (ref. 6). The pancreatic CCK nerves reported here correspond to the latter type.

Because of the high frequency of diabetes mellitus in man, the pancreatic CCK nerves are of considerable clinical interest. We have previously observed that CCK-4 is a potent insulin releaser in man¹⁰, suggesting that human islet cells may also possess receptors for CCK-4. As CCK-4-like peptides also occur in the human central nervous system and gut^{2,28,32}, it is likely that CCK-4 may also act as a neurotransmitter or neurohormone in the human pancreas. In view of the potent effect of CCK-4 on insulin secretion and possibly also on β -cell growth^{34,35} it may be

that CCK-4 or a suitable analogue may be of value in the treatment of insulin-dependent diabetes mellitus.

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LETTERS

The unusual supernova remnant CTB80

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The low latitude ($b = 2.8^\circ$) radio source CTB80 (ref. 1) is a probable, albeit unusual, supernova remnant² (SNR). A compact high brightness region with a very flat spectrum dominates its centre, and we find optical emission associated with this feature. Here we discuss evidence that CTB80 may be young, and in particular the possibility that it was observed in China at the beginning of the fifteenth century.

CTB80 is seen to best advantage in Fig. 1, a photographic representation of the 49-cm map made with the Westerbork Synthesis Radio Telescope (WSRT). Its radio emission is clearly the result of synchrotron radiation: the spectrum is nonthermal and large areas of the source are polarised. As unusual as the irregular morphology may seem, the most remarkable feature is the bright central peak with its flat radio spectrum. Notwithstanding an estimated spectral index $\alpha \approx 0$ between 49 and 6 cm, the fact that it is significantly polarised establishes its non-thermal nature.

We find that the nebulosity is most prominent on the E (or 'red') Palomar Sky Survey plate coinciding with the flat spectrum peak (Fig. 2). The agreement in both position and extent of the optical emission and flat spectrum radio component makes it highly probable that they are related. The only other nebulosity which might be associated with CTB80 is a narrow filament located some 10 arc min away from the prominent features, roughly along and aligned with the northern edge of the south-west radio ridge.

The lack of detailed radio-optical coincidence, which may be a feature of young SNR³, suggests that CTB80 could be of recent origin. Another, probably more direct indication of recent activity is the central flat spectrum component which with its morphology is somewhat reminiscent of the Crab Nebula⁴, or 3C58 (ref. 5). There have been several recent attempts^{6,7} to define and investigate the centre-filled or 'plethoric'⁸ SNRs as a separate class. Although CTB80 is more like G326.3–1.8 (ref. 9) than other members of this class, comprising as it does both compact, flat and extended, steep spectrum emission, it is perhaps worth considering whether it, too, might belong to the group. Weiler and Panagia⁷ argue that such SNRs are maintained for a relatively short period of time (10^4 yr) by a pulsar as in the Crab itself, after which their flat spectrum emission dies rapidly away. However, there does not seem to be a realistic pulsar candidate for CTB80: PSR1946+35 and 1952+29 lie too far away, about 3° north and south of it, respectively¹⁰, and a recent observation detected no scintillating component¹¹.

As far as the age of CTB80 is concerned, the purely morphological evidence is not decisive. While the irregular outer structure might suggest an old, well expanded object interacting with its environs, it could be argued that this is indicative of very recent activity, the form of the extended radio emission still dominated by the dynamics of the outburst which produced it. As noted above, the lack of corresponding optical nebulosity argues against an extremely old remnant with much swept-up material.

Table 1 Positions relevant to the new star of 1408

Object	α (1950.0)	δ (1950.0)
17 Cyg (south-east end of Niandao)	19 h 45 min	+33°37'
CTB80 nebulosity	19 51	+32 45
Cyg X-1	19 56	+35 04

But it is probably the compact, central component, whose flat spectrum suggests relative youth, which holds the key to this mysterious object. The fact that its spectrum shows no high frequency cut-off to 5 GHz enables us to set an age limit, depending on the magnetic field strength. For an equipartition field the value obtained, not greater than a few times 10^4 yr, is

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not especially valuable. If its age is 10^3 yr, then assuming that obscuration does not prevent our observing nebulosity near the centre of CTB80, there is a slight possibility that there is an historical record of the supernova. We have therefore examined a list of pre-telescopic supernova candidates compiled by Clark and Stephenson¹², and find that of their 75 events, only one is within 40° of CTB80.

Li Qi-bin¹³ has recently corrected the date of this guest star to 24 October 1408, shows that there may be nine Chinese references to it, and convincingly argues that it was a supernova. Li also suggests that it was the progenitor of the X-ray source (and black hole candidate) Cyg X-1, while we feel that an association with CTB80 is at least as likely. The only meaningful position, given in three of the chronicles, relates the new star to the Chinese constellation Niandao. This linear asterism runs from north-west to south-east, terminating at the latter end in 17 Cyg. A literal interpretation of the (translated) text, "to the southeast of Niandao", suggests a position both south and east of 17 Cyg (like that indicated by Clark and Stephenson¹²), consistent with CTB80 but excluding Cyg X-1 (Table 1). A looser interpretation of the wording would probably include anything within several degrees of 17 Cyg, and hence both candidate objects.

An indirect reason for preferring an association between CTB80 and the star of 1408 is that on both observational and theoretical grounds we expect supernovae to produce SNRs, and Cyg X-1 has no associated extended radio emission. One also wonders whether a supernova explosion would not have disrupted the binary system to which Cyg X-1 belongs. Although these arguments are somewhat weakened by the fact that we do not know what the event responsible for Cyg X-1 might have looked like, we will continue our discussion assuming that an association with CTB80 is more likely. What are the consequences of this assumption?

We concur with Li's conclusion that the star of 1408 must have been bright. (In addition to the reasons he gives, note that the description in the 'Ming Shilu' means that the star was probably observed just after sunset and before the end of

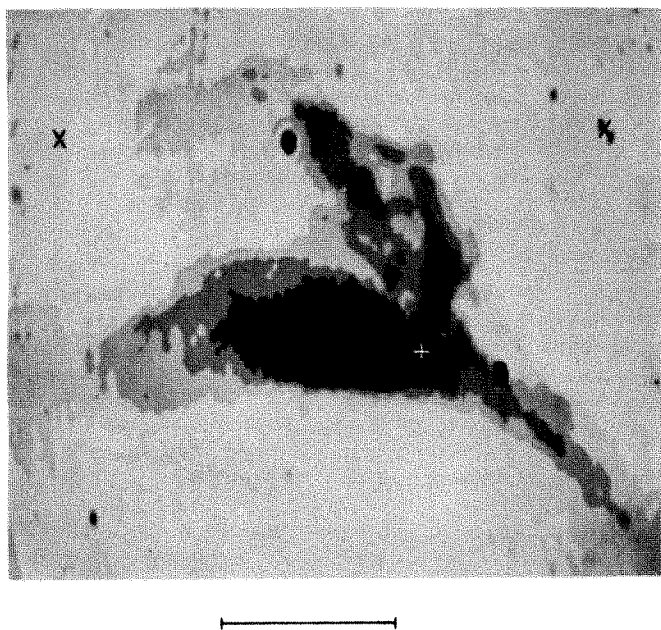


Fig. 1 A shaded contour photographic representation of the brightness distribution observed in CTB80 at 49 cm wavelength made with a 1 by 2 arc min beam. +, The highest peak, a flat spectrum compact component at the western end of the plateau; x, the positions of two strong sources which have been subtracted. North is at the top, and centre is $\alpha = 19^{\text{h}} 51.6^{\text{m}}$, $\delta = 32^\circ 49'$. Scale bar, 20 arc min.

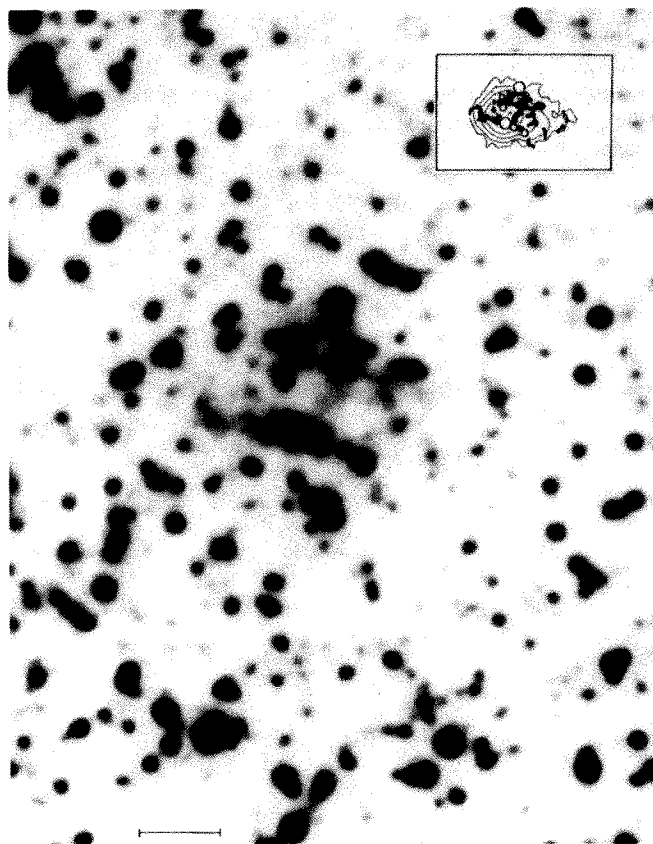


Fig. 2 An enlargement of the Palomar Sky Survey E plate (Copyright National Geographic Society-Palomar Sky Survey), showing the main nebulosity near the centre of CTB80. In the inset the optical emission has been sketched on a contour plot of the 6 cm map of the flat spectrum component made with a 7 by 13 arc s beam. The most prominent optical feature is the knot of emission near the western edge, elongated in position angle 80° . From prints made with various exposures it is clear that there are no sharp emission features and that although diffuse nebulosity probably fills the entire radio emitting region, there appears to be a hole in the centre. Scale bar, 20 arc s.

astronomical twilight.) The chronicles consistently describe it as 'like a lamp', wording similar to that used for the supernova of 1572 ('large as a lamp') when its apparent magnitude was -3.5 mag (ref. 12). The lack of European reference to the 1408 star suggests that it was not as bright as the supernova of 1006 which did arouse interest¹². We conclude that the peak magnitude was probably -4 ± 1 mag. In a recent optical and radio investigation, Angerhofer *et al.*¹⁴ conclude that the likely distance to CTB80 is 3 ± 1 kpc, with at least 3–4 mag of absorption. If the maximum absolute magnitude of the supernova was -19 mag we would expect an apparent magnitude of -3.1 mag, in reasonable agreement with our above estimate for the 1408 star.

The spectral data obtained by Angerhofer *et al.*¹⁴ are consistent with a shell expanding at 35 km s^{-1} . If the distance (3 kpc) and age (570 yr) suggested above are correct, the shell has expanded at an average speed of 375 km s^{-1} . This is not irreconcilable with the spectral data if there has been substantial deceleration. Alternatively, the nebulosity may be excited circumstellar gas like the quasi-stationary flocculi in Cas A (ref 15).

Average velocities required in the outermost parts of the radio source are substantially higher, however. The optical wisp south-west of the main nebulosity must have travelled at some $13,000 \text{ km s}^{-1}$ since 1408, and the speed of material at the very edges of the radio emission would have to be at least four times higher. All of this assumes that a single outburst was the cause of CTB80 in its entirety, which may not be a necessary condition.

Apart from the possibility that we are seeing two totally unrelated sources juxtaposed (which we consider quite unlikely), we have no evidence which contradicts the notion that the outer arcs and ridges are the result of activity before 1408.

Despite the substantial body of data collected there is clearly much work to be done if we are to achieve a good understanding of this perplexing object. It does seem plausible that CTB80 is a young supernova remnant located within a few kiloparsecs of the Sun. Although it exhibits a superficial similarity to remnants like the Crab Nebula, its morphological and other properties make it unique among galactic nonthermal objects.

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Photoelectrochemical properties of metalloporphyrins

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The photovoltaic responses of *meso* tetraphenyl-, *meso* tetrapropyl- and octaethyl-porphyrins, porphines, chlorins, cofacial diporphyrins and mesoporphyrin IX diesters were investigated using two cell configurations:

Al|Porphyrin|Ag, and Al|Porphyrin|Fe(CN)₆³⁻, Fe(CN)₆⁴⁻|Pt.

We found (1) that the Al–porphyrin interface is photoactive: the action spectra closely follow the absorption spectra of the porphyrins, and this interface is best described as a semiconductor–insulator–metal diode consisting of porphyrin|Al₂O₃|Al; (2) that within a homologous series, in which the porphyrin skeleton is fixed but the metal is varied, the quantum yields parallel the ease of oxidation of the porphyrin in nonaqueous solvents. The more easily oxidised compounds exhibit the higher quantum yields; (3) no obvious correlations are found with the luminescent properties of the porphyrins in solution; (4) the morphology of the films influences the quantum yields: amorphous films are better than microcrystalline ones; and (5) the most efficient cells reach quantum yields of ~0.2 and energy efficiencies of ~1% for monochromatic light at the peak of the action spectrum in the region of 400–450 nm.

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Although attempts to build photovoltaic and photoelectrochemical devices sensitised by dyes have met with varying degrees of success^{1–14}, the high quantum yields and energy efficiencies recently reported^{15–17} for the primary photoprocesses in photosynthetic organisms have stimulated a renewed interest in artificial systems based on chlorophyll derivatives. The inherent chemical fragility of chlorophyll and bacteriochlorophyll *in vitro* has focused attention on synthetic porphyrins, whose physical and chemical properties can be readily tailored by varying their metal and/or their organic framework. Examples of these are the recent reports of photovoltaic cells based on phthalocyanine^{18,19} and porphyrin²⁰ films sandwiched between metals of low work functions, such as aluminium or indium, and metals of high work function, silver or gold. These cells generate open circuit voltages (V_{oc}) of several tenths of a volt with short circuit currents of microamperes, and quantum

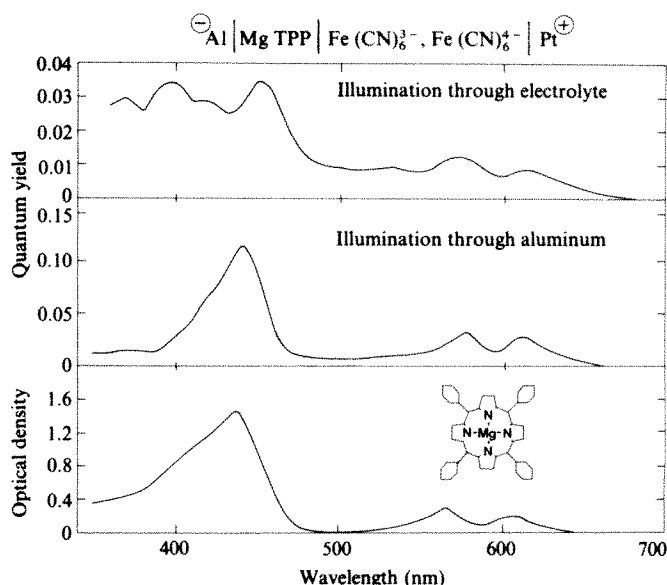


Fig. 1 Comparison of the optical spectrum of a magnesium tetraphenylporphyrin film with the action spectra, obtained for the cell shown, on illumination of the Al/porphyrin and Pt/electrolyte/porphyrin electrodes. Note the large differences in photocurrent quantum yields at the two interfaces.

yields as high as 0.1. The Al–dye (or In–dye) interfaces exhibit rectifying behaviours attributed to Schottky barriers induced by interaction of the p-type semiconductor dyes with the metals. A similar cell, which used zinc tetraphenylporphyrin layers on Al and a solution of ferri–ferro cyanide as ohmic contact, was reported by Wang²¹ to generate $V_{oc} > 1$ V and two such cells in series oxidised water to oxygen.

To assess the effects of metal and substituents, we have screened the photoresponses of a large number of porphyrin derivatives.

Figure 1 compares the photocurrent quantum yield obtained for a sublimed film of magnesium tetraphenylporphyrin (MgTPP) in a Wang cell with the optical spectrum of the porphyrin film. The large differences in photocurrent obtained on irradiation of the two electrodes and the similarity of the action spectrum at the Al electrode to the absorption spectrum of the porphyrin, clearly indicate that the Al–porphyrin interface is the photoactive region. Similar results are obtained with films deposited by rapid evaporation of concentrated solutions of dyes except that the absorption bands of the films are less diffuse and resemble solution spectra. Although the Al–porphyrin interface has been characterised²⁰ as a Schottky

barrier, the dark current-voltage behaviour of the cells suggests that the electrode assembly exhibits the properties of a metal-insulator-semiconductor diode²² (Fig. 2).

The significant effects of ligand and metal variations are illustrated in Table 1. Peak quantum yield (calculated for short circuit currents obtained on illumination through the semi-transparent aluminium electrode at the Soret absorption band of the porphyrins) ranged from 10^{-1} to 10^{-4} at wavelengths between 400 and 450 nm. Films prepared by rapid evaporation of solutions using a photoresist spinner were ~50% more effective than films prepared by sublimation. The latter were found to be microcrystalline by electron microscopy at 20,000-fold magnification while the former appeared amorphous.

For a given porphyrinic ligand, the quantum efficiencies of the photocurrents paralleled the first oxidation potential of the porphyrin (Fig. 3) but all porphyrins did not fall on the same curve. Octaethylporphyrins, for example, were less efficient than other porphyrins. This may be due to a combination of packing, extent of overlap that facilitates exciton migration, and the property of this class of porphyrins to form dimeric dications²³ on oxidation which may act as electron traps. The significant effects of substituent groups on the photovoltaic properties were especially evident in the poor response of the mesoporphyrin IX diesters where the long aliphatic chains may

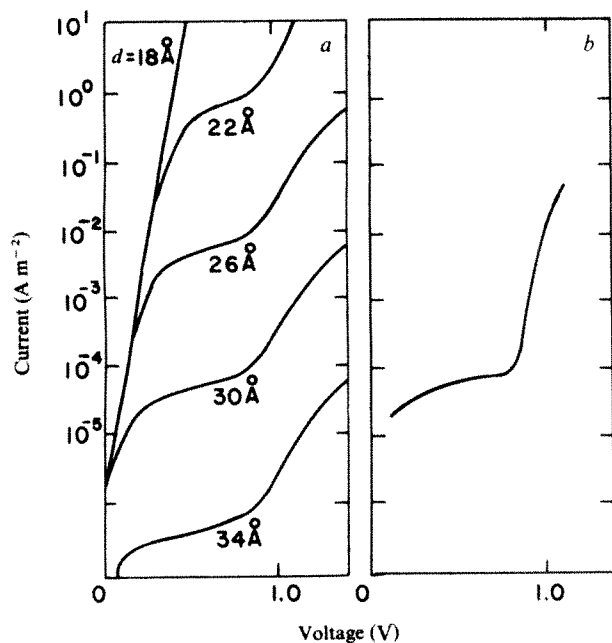


Fig. 2 Forward bias, current-voltage curves for a silicon, metal-insulator-semiconductor junction (a) and a porphyrin photoelectrochemical cell (b). a, A simulation of an Al/SiO₂/p-type Si/ohmic contact for different SiO₂ thicknesses²². b, The characteristics of an Al/MgTPP/Fe(CN)₆³⁻, Fe(CN)₆⁴⁻/Pt cell.

prevent good π - π overlap between the porphyrin rings and act as insulators towards electron transport. Although dimeric chlorophylls and bacteriochlorophylls are thought^{16,17} to act as the phototaps and primary donors in photosynthetic systems, synthetic diporphyrins were less effective than monomers in the cells considered here.

No obvious correlations were discerned between quantum yield and the luminescent properties of the porphyrins in solution. The platinum and palladium complexes, which decay²⁴ exclusively from excited states via triplets were not exceptional

Table 1 Photocurrent quantum yields at the wavelength of maximum absorption for the cell shown as a function of porphyrin and metal

Compound*	λ_{\max} (nm)	Quantum yield†
Sublimed films/Wang cell‡		
MgP	420	2.0×10^{-1}
CdP	410	1.6×10^{-1}
MgTPP	445	9.8×10^{-2}
ZnP	420	9.1×10^{-2}
MgTPC	440	5.2×10^{-2}
ZnTPiBC	425	4.5×10^{-2}
CdTPP	450	4.1×10^{-2}
ZnOEC	405	3.5×10^{-2}
CdOEP	410	2.8×10^{-2}
ZnOEP	400	2.5×10^{-2}
CdT(n-Pr)P	435	2.2×10^{-2}
MgT(n-Pr)P	445	2.1×10^{-2}
MgOEP	420	1.5×10^{-2}
MgOEC	420	1.4×10^{-2}
H ₂ T(n-Pr)P	435	1.4×10^{-2}
ZnTPC	435	1.3×10^{-2}
PdOEP	405	1.3×10^{-2}
PtOEP	390	1.0×10^{-2}
ZnTPP	440	6.1×10^{-3}
ZnT(n-Pr)P	425	5.2×10^{-3}
PdT(n-Pr)P	430	3.9×10^{-3}
PbT(n-Pr)P	475	3.8×10^{-3}
H ₂ OEP	415	3.4×10^{-3}
H ₂ TPC	440	2.4×10^{-3}
CuT(n-Pr)P	420	2.0×10^{-3}
PdTPP	440	1.1×10^{-3}
H ₂ TPP	445	7.5×10^{-4}
CuTPP	430	6.6×10^{-4}
CoTPP	430	1.2×10^{-4}
PtTPP	430	7.3×10^{-5}
Spun films/Wang cell		
CdTPP	453	2.7×10^{-1}
MgTPP	445	1.7×10^{-1}
ZnTPP	445	5.0×10^{-2}
MgOEP	415	3.6×10^{-2}
Mg-Mg-7	417	2.5×10^{-2}
H ₂ TPP	440	1.8×10^{-2}
Mg-H ₂ -7	430	1.2×10^{-2}
Spun films/sandwich cell		
MgTPP	450	1.0×10^{-1}
ZnTPP	440	4.0×10^{-2}
MgOEP	425	4.0×10^{-2}
PdOEP	410	3.3×10^{-2}
Mg-Mg-7	415	2.1×10^{-2}
MgMPDHE	425	2.0×10^{-2}
MgMPDOE	425	4.4×10^{-4}

* Abbreviations used: MPDHE, mesoporphyrin dihexylester; MPDOE, mesoporphyrin dioctadecylester; OEC, octaethylchlorin; OEP, octaethylporphyrin; P, porphine; T(n-Pr)P, tetra(n-propyl)porphyrin; TPC, tetraphenylchlorin; TPiBC, tetraphenylisobacteriochlorin; TPP, tetraphenylporphyrin; Mg-Mg-7 and Mg-H₂-7, cofacial diporphyrins with seven atoms in the covalent linking chain. See ref. 25.

† Quantum yield is defined as electrons produced per photon incident on the porphyrin film at low light intensities. Because of the high optical density of the films at the wavelengths used (absorbances between 1 and 2), the number of photons absorbed is nearly equal ($\pm 10\%$) to the number of photons incident on the film.

‡ Film thicknesses of $\sim 0.1 \mu\text{m}$ were monitored with a Kronos QM331 quartz crystal oscillator.

compared to porphyrins with lower triplet yields. An attempt to alter the lifetime of the porphyrin triplet by completely deuterating MgTPP, yielded the same results as the protonated form.

In the Wang cells, the power efficiencies varied from 1 to $10^{-4}\%$ for monochromatic light in the 400–450 nm region. The best results were obtained with magnesium tetraphenylporphyrin and cadmium porphyrin which yielded open circuit voltages of ~ 1 V, quantum yield of ~ 0.2 , and $\sim 1\%$ power efficiencies.

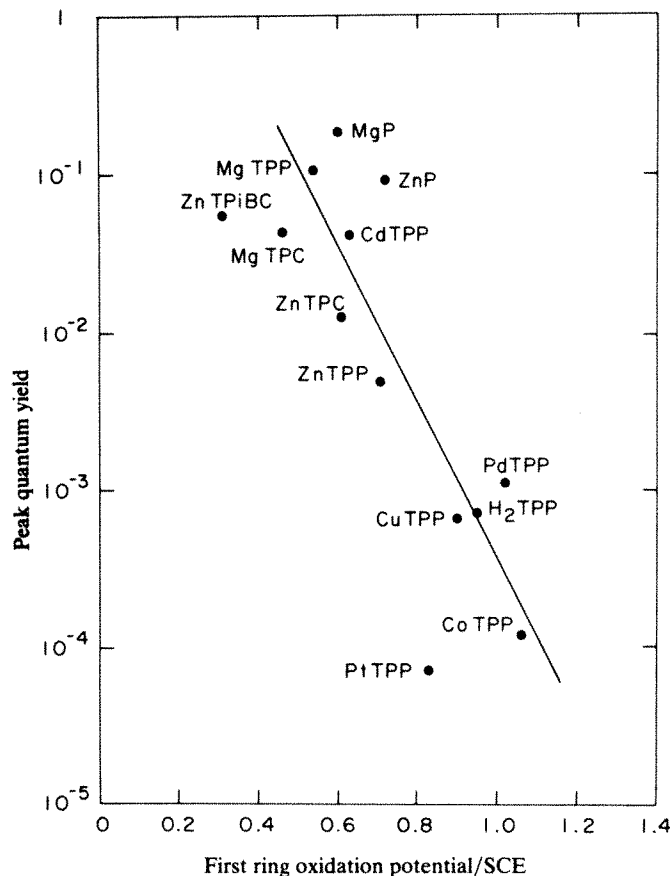


Fig. 3 Correlation between quantum yield and the first oxidation potential (versus SCE) of porphyrins in non-aqueous solvents. The line shown represents a least squares fit to the tetraphenylporphyrin data excluding PtTPP. (The latter is omitted because films of PtTPP on Al surfaces show unusually sharp absorption bands not observed on glass surfaces. A specific Pt-Al interaction may therefore account for the low quantum yield of PtTPP.)

The effects of film morphology, the use of mixtures of porphyrins, of other metals of low work function and of semiconductor electrodes are being investigated.

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Spatial distribution studies of thermoluminescence using a high-gain image intensifier

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Thermoluminescence (TL)—the light emitted when a previously irradiated material is heated—is normally studied by heating the material under a photomultiplier (PM). This measures the total light emitted within the acceptance angle of the PM, but provides no information on the spatial distribution of the TL. The dating of archaeological specimens by means of their naturally acquired TL is a common application of this method. While such a spatially-averaging technique is acceptable in studying TL from homogeneous materials, problems can arise when it is applied to inhomogeneous materials, such as certain natural calcites¹, where TL emissions may be correlated with internal irradiation dose rates. As we shall demonstrate, a high-gain image intensifier (IIT) can be successfully used to investigate the spatial distribution of TL from such materials at light levels where even fast photographic emulsions fail to record any emission. So far as we are aware this is the first time that high-gain image intensification techniques have been used in such studies.

The equipment, shown in Fig. 1, is centred on an EMI type 9914 intensifier. This is a magnetically-focused four-stage tube (of phosphor-photocathode sandwich construction) with a bialkali input and a P11 output phosphor. At an EHT setting of 40 kV the light gain of the tube is around 10^6 . This is sufficient to ensure that approximately 65% of the photoelectrons leaving

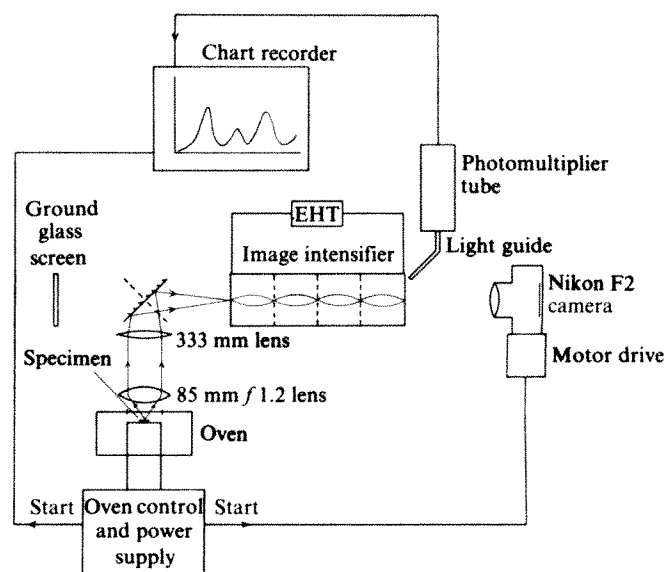


Fig. 1 Schematic diagram of the IIT and associated apparatus used to record the spatial distributions of TL emissions from a heated specimen in given temperature intervals together with the corresponding glow curve.

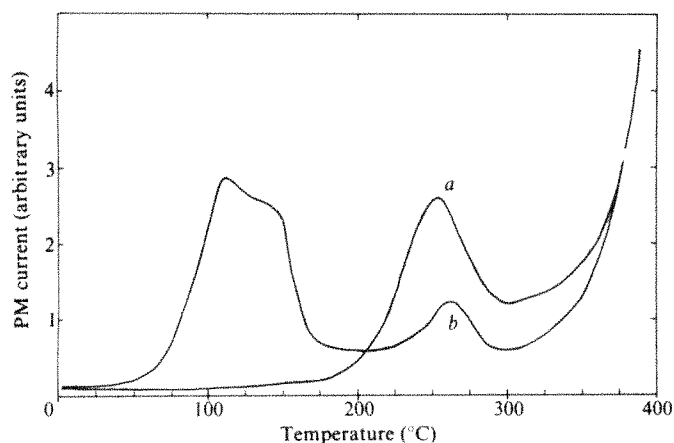


Fig. 2 Glow curves obtained from a sample of natural calcite obtained from a palaeolithic cave at Pont-Newydd, Wales. The specimen was heated linearly with time while a chart recorder traced the current from a photomultiplier tube which viewed the output phosphor of the IIT. Curve *a* shows the naturally acquired TL, and *b* the TL induced by an artificial γ irradiation of 12 krad.

the input photocathode (peak quantum efficiency 18%) are recorded as discrete 'points' on the film (Kodak Tri-X) taken in the camera viewing the output phosphor of the tube. (The camera is a motor-driven Nikon F2 with an *f*1.2 lens and a 4 dioptr achromatic doublet as a close-up lens.) The specimen is imaged onto the input photocathode using a microscope comprising a Canon 85 mm *f*1.2 photographic lens placed 'back-to-back' against a single-element lens of 333 mm focal

length, giving a linear magnification of 3.9. A rotating mirror allows the specimen to be brought into focus on either a ground glass viewing screen or the input photocathode. The advantages of such a microscope over a conventional one are the high aperture realised at low magnifications and the long working distance between the specimen and the 'objective' lens. The latter feature is important since the specimen is heated in an oven containing a 5-mm thick Spectrosil window and a 5-mm Chance-Pilkington HA-3 IR absorbing filter. The oven consists of an electrically-heated nichrome plate whose temperature, recorded by means of a NiCr/NiAl thermocouple, can be increased linearly with time². Starting the temperature ramp activates both the camera's motor drive and the time base of a chart recorder. The ordinate of the chart record shows the anode current of a PM viewing uniformly the total area of the output phosphor of the IIT; the resulting graph is conventionally called a glow curve.

In these studies we have used the intensifier system to observe the light emission from naturally occurring calcite of late Pleistocene age. Preliminary attempts to record even saturated TL in calcite using 3000 ASA film at 1:1 magnification had proved unsuccessful. Specimens were prepared in the form of 500 μ m thick slices. The surfaces were etched for 1 min in 1% acetic acid to eliminate spurious emission induced by the cutting of the slices³. Each specimen was first heated to observe its naturally acquired TL. The drained specimen was then given a γ irradiation of 12 krad and the artificially induced TL was observed in a second heating. Figure 2 shows the resulting glow curves for a sample of calcite (~10 mm across) obtained from a palaeolithic cave near Pont-Newydd in Wales. (The material from which the sample was prepared is stalagmitic, containing 1% of detritus, insoluble in HCl. Its mean ²³⁸U concentration is 0.5 p.p.m.) Figure 2*a* shows the natural TL and Fig. 2*b* the artificial TL. The

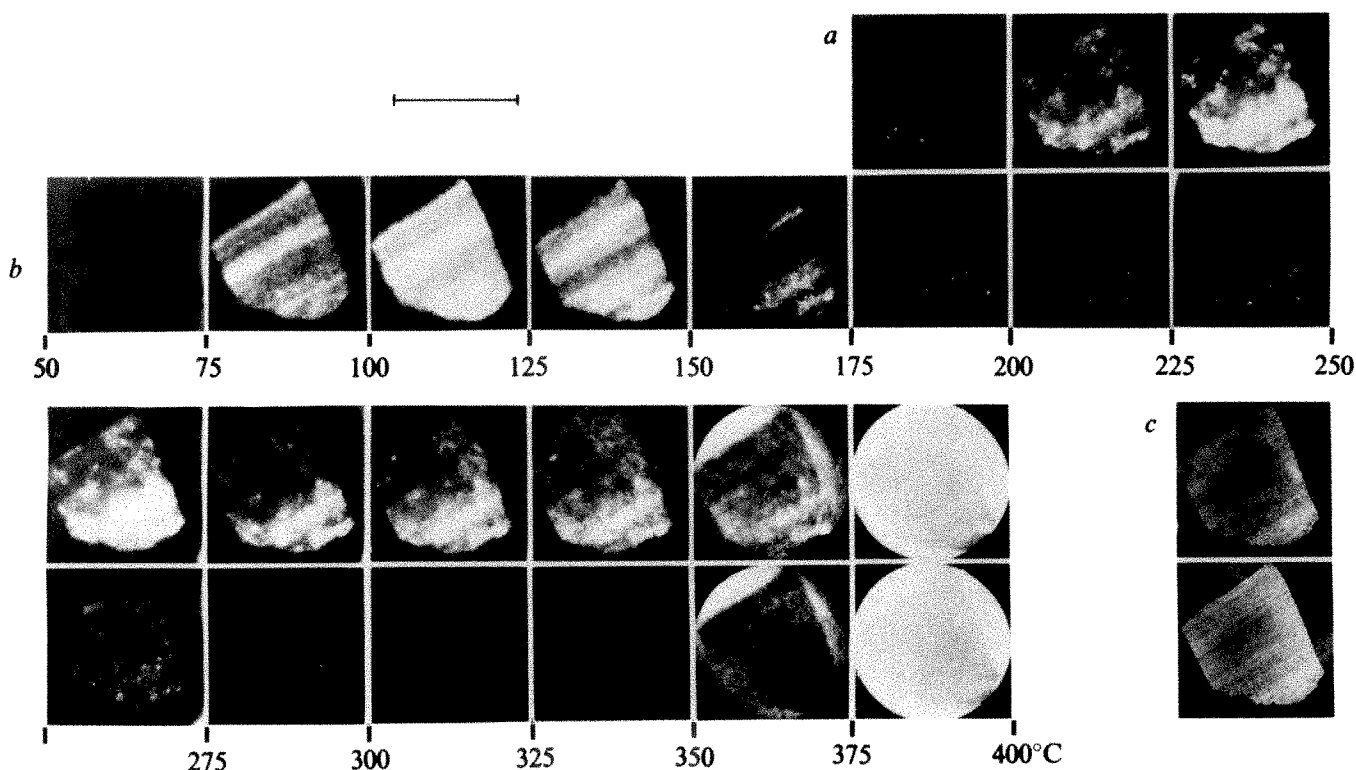


Fig. 3 Photographs of the output phosphor of the IIT showing spatial distributions of TL emissions from a specimen of natural calcite approximately 10 mm across on two subsequent heatings. Sequences *a* and *b* were taken concurrently with the respective glow curves *a* and *b* shown in Fig. 2. That is, *a* records the naturally acquired TL of the specimen, and *b* an artificially induced TL. Each frame was exposed for 10 s, and correspond to temperature intervals of 25 °C. (Frames in the sequence of natural TL below 200 °C are not reproduced as they recorded no light emission.) The 85-mm microscope lens was stopped at *f*1.2, and the Nikon camera lens at *f*1.2. The images recorded on the 35 mm Kodak Tri-X film were given standard development for a γ of 0.7 in Promicrol developer. All frames were given the same printing exposure. Photographs in *c* were taken at the end of each sequence of the cooled specimen under low-level illumination: a blue filter was used at the input end to enhance contrast. For these exposures both the microscope and camera lenses were stopped down to *f*5.6. Scale bar, 10 mm.

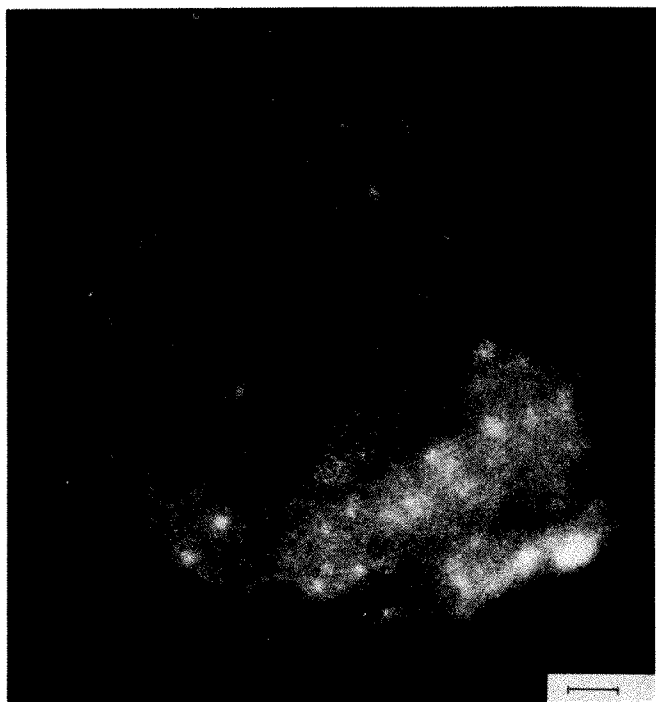


Fig. 4 Reproduction of the frame in sequence *a* of Fig. 3 corresponding to the temperature interval 275–300 °C, showing in more detail the intense ‘spots’ of TL emission against a more uniform, though banded, background. Scale bar, 1 mm.

corresponding sequence of photographs are shown in Fig. 3*a* and *b*. The temperature ramp rate was 2.5 °C per s and each frame was exposed for 10 s. Thus each frame records the integrated light emission within a 25 °C interval. Figure 3*c* shows the specimen photographed through the IIT under a low level of reflected light. (This procedure was adopted as conventional photomicrographs fail to reproduce geometrical distortions introduced by the IIT.) The glow curves shown in Fig. 2 are typical of those obtained by conventional PM techniques for many natural calcites. The peak at around 270 °C is commonly used in TL dating studies.

A striking feature of the sequences of photographs shown in Fig. 3 is the degree of non-uniformity in the light emission from both the natural and the artificial TL. (Frames in the sequence of natural TL below 200 °C are not reproduced as they recorded no light emission.) Looking at the sequence of natural TL emission (Fig. 3*a*) we see that most of the light emitted at the glow curve peak near 270 °C (Fig. 2*a*) in fact comes from localised ‘spots’ situated mainly in the lower half of the specimen. These spots may be seen more clearly in Fig. 4 which reproduces on a larger scale, the natural TL emission in the interval 275–300 °C. Their diameter is less than 0.5 mm and they appear against a more uniform emission. The glow curve of the artificial TL emission (Fig. 2*b*) shows a composite peak at around 125 °C in addition to the 270 °C peak. As can be seen from the sequence of photographs (Fig. 3*b*) the low-temperature emission is strongly banded with a few spots also contributing to the overall emission. Although the less intense emission at around 270 °C is more uniform it does show evidence of banding (particularly on the photographic negatives). The spots contribute a far smaller proportion of the high temperature emission in the artificial than in the natural TL. However, there is also evidence in the negatives of banding in the natural TL. Note that the growth layers visible in Fig. 3*c* are parallel to the banding seen in Fig. 3*a* and *b*. The bright background seen in the last two frames (375–400 °C, 400–425 °C) of each sequence is due to black-body emission from the heater plate.

There are at least two ready explanations for the ‘spots’ evident in the natural TL emission (Fig. 3*a*). The most likely one

is that they are associated with the detrital inclusions, insoluble in dilute HCl, which are present as 1% of the material. These mineral inclusions would be of geological age and likely to contain relatively high concentrations of uranium. Another possibility is that the spot emission originates from particles of limestone within the calcite matrix, the intensity of emission being mainly the result of their geological age. The apparent range of spot sizes (Fig. 4) is due to the varying depths of the emitting centres within the slice and the resulting variations in scatter of the emerging light. Observations on calcite samples from another site (Caune de l’Arago, France) have broadly confirmed the two basic features of spots and banding; the former being the predominant source of TL in young samples, the latter the predominant source in old samples (>200,000 yr). Adopting the methods of Fremlin and Srirath⁴ we have made fission-track maps of the old-age material and from these initial observations we have found that the uranium tends to be concentrated in the growth layers rich in iron impurities. These same impurity bands are also found to have higher optical attenuation coefficients. In addition the material’s TL sensitivity to irradiation is believed to vary between the different growth layers. It is a combination of these three effects which produces the banding structure which we have observed in the natural TL, while the last two factors are relevant in the case of artificial TL. Further studies are in progress on the spatial distributions of the TL and uranium in calcite with the ultimate aim of improving the reliability and accuracy of TL dates. Although still at an early stage, image intensification techniques already seem to provide a powerful tool for the study of TL in inhomogeneous materials.

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Surface potentials of ice and thunderstorm charge separation

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There is no general agreement on the nature of the mechanism which causes thunderstorms to become electrified^{1,2}. Many scientists believe that charge is transferred when ice crystals collide with hailstones, and that subsequent gravitational separation results in field growth to breakdown magnitudes. Buser and Aufdermaur³ have proposed that the charging results from differences in the surface potential of ice. The measurements described here suggest that the surface potential of polycrystalline ice, such as would occur in a thunderstorm, is related simply to its past growth history. The sudden step in potential postulated by Buser and Aufdermaur as the ice surface changes from an evaporating to a growing state could not be detected. However, a natural hailstone grows by riming as it collects supercooled droplets, and these new measurements suggest that this effect results in much larger changes in surface potential.

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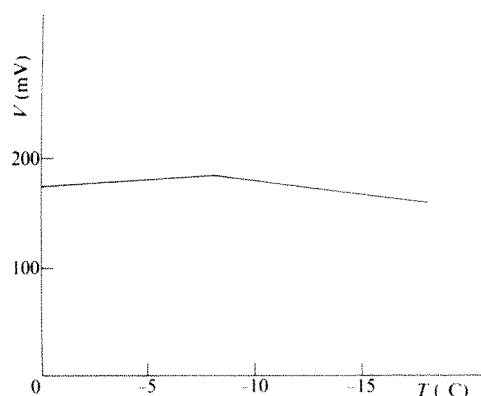


Fig. 1 Variation of the surface potential, V , of ice using gold electrodes with air temperature, T . The maximum potential always coincided with the ice temperature which was -8°C in this example; to the left the potential falls (ice cooler than air and condensing) and to the right it also falls as the warmer ice is evaporating.

Laboratory experiments which attempt to measure the charge separated when an ice crystal collides with a simulated hailstone, have not yielded consistent or predictable results. Possible clarification of this confusion is provided by Buser and Aufdermaur who found that when an ice crystal collided with various metal surfaces at -45°C the charge separated was proportional to the difference in the work function of the substances. Ice impacting on ice gave more variable results but if the target was evaporating it acquired negative charge whereas after deposition positive charge was favoured and the difference in charge per collision was equivalent to a change in the surface potential of the order of 0.2 V. This agreed with Takahashi's⁴ measurements of changes in the apparent surface potential of a copper electrode when ice was condensing on it or evaporating from it, and changes in the surface potential of a single crystal of ice also growing by deposition or evaporating at -10°C (ref. 5). The implicit assumption being that the surface potential change occurs in a single step. However, Buser and Aufdermaur concluded that the surface potential can be modified by so many factors that it may be difficult to predict in natural situations.

We report measurements of changes in the surface potential of ice made using a vibrating capacitor technique similar to that of Takahashi⁵. A cylinder of polycrystalline ice 10 mm thick and 50 mm diameter formed from distilled water was attached to the lower noble metal electrode by heating the electrode to near 0°C and warming the surface of the ice with hot air. The lower electrode was mounted on a Peltier element so that the specimen could be warmer or cooler than the ambient air. The upper electrode was made of gold plated gauze to allow an equilibrium vapour flux to the surface; if a solid electrode was used the results were variable and dependent on ventilation.

Figure 1 shows the uniform change in surface potential of about $1.5\text{ mV}^\circ\text{C}^{-1}$ as the temperature of the air was varied whilst keeping the ice and metal substrate at a constant temperature. The change in the surface as it evaporated or grew by deposition was clearly visible to the eye, but was not associated with any sudden change in potential. The upper electrode was always kept 1°C warmer than the lower one to prevent frost deposit. Without this precaution potential changes showing hysteresis similar to those of Takahashi⁵ were obtained as frost formed on the gauze and then evaporated.

Similar results to Fig. 1 were obtained for different ice specimens at different temperatures and dew points and for electrodes of Au, Pt, or Pd. Changing the lower electrode altered the voltage offset but not the shape of the curves. The measurements were reversible with no hysteresis. We confirmed Hobb's⁶ statement that copper-ice electrodes give results which are unpredictable and not reproducible, but we could produce results comparable with Takahashi's⁴ if we prepared the copper electrodes in the exact manner he describes.

The most striking changes in surface potential were observed when the ice surface was rimed with supercooled droplets from an ultrasonic generator (radius $50\text{ }\mu\text{m}$, water content $1\text{--}2\text{ g m}^{-3}$) falling at terminal velocity. Figure 2 shows that the change in surface potential was a function of temperature. The ice, air and supercooled water droplets were all at the same temperature; although a subsidiary experiment showed that if the ice was a few degrees warmer than the air the same results were obtained. Identical results were obtained with $10\text{-}\mu\text{m}$ radius droplets.

If the ice was only partially covered with frozen droplets a smaller change was observed, but when further layers were added to an already complete layer no further increase in potential resulted. The potential change was reversible. If the rime on the surface of the ice was melted and then allowed to refreeze slowly, the potential returned to its original value. As soon as the melted surface was refrozen the process could be repeated with a new layer of droplets. The slow refreezing of the surface was achieved by heating the upper electrode while it was close to the ice. If a very thin surface layer of water was rapidly frozen by exposing it suddenly to a stream of cold air the same change was observed as with riming.

The changes in potential could be very persistent. If the rime was left for 24 h the potential was practically unchanged and surface melting returned it to the previous day's value. Care was taken not to confine the ice specimen mechanically around its perimeter, otherwise slow and unpredictable changes occurred as the temperature varied. This is probably due to the stress within the ice⁷. If the rimed surface of the ice was exposed to an electrical field of 500 kV m^{-1} , or a charged probe touched the surface, the potential returned to its previous value a few seconds after the field was removed.

The effect of impurities was found to be small: 10^{-4} M solutions of NaCl, NH_4OH , and HF gave the same results as Fig. 2 and rainwater gave values within 20%. These changes persisted for many hours. When the concentrations were increased 100-fold, changes were more variable and the voltage decay took only several minutes. These higher concentrations are quite unrepresentative of atmospheric conditions.

Note that during the actual freezing of the droplets of both distilled and doped water large transient potentials are developed which we interpret as Workman-Reynolds freezing potentials⁸. Because the chance of an ice crystal impacting on a hailstone at a position where a supercooled droplet is in the process of freezing is so small, these freezing potentials are not likely to be important in thunderstorms.

Previous measurements^{5,9,10} of changes in potential during riming showed a steady change in potential proportional to the riming rate, and it was postulated that charged splinters were ejected, but it is now established¹¹ that the splinter production was caused by the high concentration of CO_2 used for cooling.

The origin of this potential change during the rapid freezing of supercooled water is a formidable theoretical problem. The

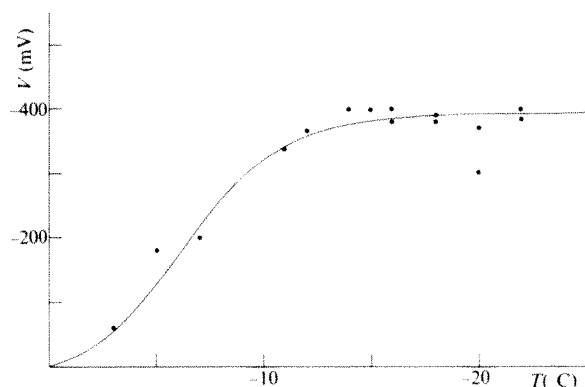


Fig. 2 Change in the surface potential, V , of ice after riming as a function of temperature, T . The unrimed ice surface is assigned a potential of 0V.

temperature dependence in Fig. 2 and the fact that rapid freezing of a very thin layer of surface water gives identical results, suggest that the rate of freezing is a controlling parameter. The freezing velocity of ice in supercooled water varies with the square of the degree of supercooling¹² which is similar in form to the first part of Fig. 2.

When ice is subject to thermal stress so that cracks appear, potentials up to 40V have been measured⁷ which decay rapidly and in a temperature dependent manner. The potentials developed in these experiments resulting from the rapid freezing of supercooled water were two orders of magnitude less and only decayed rapidly if the ice was heavily doped or subject to external stress.

The large differences in surface potential between a rimed and a non-rimed ice particle should be a more powerful mechanism for separating charge when ice crystals collide with hailstones in thunderstorms than the much smaller potentials existing between evaporating and condensing ice surfaces.

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Allophane isolated from a podsol developed on a non-vitric parent material

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The relationship between allophane, an X-ray amorphous, short-range ordered aluminosilicate gel, found in soils derived from volcanic ash (Andosols) and the poorly ordered clay minerals present in the subsoils of podsoles (Spodosols) developed on non-vitric parent materials is uncertain. The similarity of many of the physical and chemical properties of these soils has led to postulations that 'allophane-like' materials also occur in podsoles^{1–4}. The high chemical reactivity of allophane and its intimate association with both soil organic matter and crystalline phyllosilicates and oxides has meant that earlier studies have involved either the examination of mixed crystalline–microcrystalline clay systems, or the use of dispersing reagents that would have caused at least the partial solution of any poorly ordered phases present. The established instrumental techniques of differential thermal analysis and IR absorption spectroscopy may be used to characterise allophanic materials, but only when they constitute virtually the whole of the clay fraction⁵. The present study characterises an allophane sample successfully isolated from a podsol profile.

The close similarity of the properties of naturally occurring soil allophanes and synthetic aluminosilicate gels^{6,7} suggests that the former are likely to have formed by precipitation from solution. If such a solution mechanism is involved, a vitric parent material should not be an essential requirement for allophane formation. The dominance of vitric parent materials in studies

involving soil allophanes, and the comparative paucity of convincing observation of allophane derivation from other parent materials⁸, may simply reflect the difficulties involved in isolating and characterising allophane when it constitutes only a small part of a clay fraction.

An opportunity to isolate and hence characterise a poorly ordered aluminosilicate gel formed during the genesis of a podsol has arisen during the study of a catena of podsolised yellow-brown earth soils on Bealey Spur, in the eastern foothills of the Southern Alps, North Canterbury, South Island, New Zealand (43°02' south latitude, 171°37' east longitude). The parent materials of the soils are mixed colluvium and locally derived loess overlying glacial till. The parent materials are all derived from rocks of the Canterbury Suite⁹. The dominant lithotype in the suite is a thick-bedded sandstone of Upper Triassic age. There are smaller occurrences of siltstones, mudstones, conglomerate, stratiform volcanics (basalts and dolerites), limestone and banded chert. The soils have developed under a forest vegetation of mountain beech (*Nothofagus cliffortioides* var. *solandri*).

Table 1 Chemical properties of the clay-sized separates of a podsol and selected allophanes

Size fraction (µm)	Podsol		Taupo B Taupo C	
	<0.2	0.2–2	<0.2	<0.2
Ignition loss 105–600 °C (%)	47.3	43.6	43.9	27.7
Organic carbon (%)	15.3	14.2	13.2	6.3
Total Si (%)	13.9	16.7	17.5	21.6
Total Al (%)	32.4	28.1	25.4	21.7
Total Fe (%)	1.2	1.9	7.9	6.0
SiO ₂ /Al ₂ O ₃ mole ratio	0.82	1.1	1.3	1.9
Oxalate–Si as % total Si	67	39	28	14
Oxalate–Al as % total Al	94	90	87	71
Oxalate–Fe as % total Fe	41	19	92	78
SiO ₂ /Al ₂ O ₃ mole ratio (extracted)	0.58	0.47	0.44	0.38
NaOH–Si as % total Si	76	57	76	68
NaOH–Al as % total Al	98	90	90	79
SiO ₂ /Al ₂ O ₃ mole ratio (extracted)	0.64	0.72	1.1	1.6
OH [−] released in 25 min by 0.85 M NaF at pH 6.8 (mmol g ^{−1})	39.8	32.9	23.9	22.2

Results are expressed on an ignited mass basis except for ignition loss and organic carbon which are on an oven-dry basis.

X-ray and microscopic examination of the sand fractions of the catena soils show them to be composed largely of quartz, altered feldspars and rock fragments consisting of quartz particles in a strongly weathered matrix. Minor to trace amounts of muscovite, biotite, chlorite, hornblende and garnet occur, particularly in the less weathered lower horizons. No fragments of volcanic glass could be detected.

The only minerals that could be determined in the silt fractions of the catena soils were quartz, feldspar, mica and chlorite. The clay fractions were dominated by phyllosilicate minerals derived from mica and contained minor amounts of gibbsite, kaolinite, allophane and quartz. Thermal and selective dissolution analyses indicated that allophane occurred widely throughout the catena. The greatest amounts of allophane and of gibbsite occurred in the lower part of the B horizons.

In the toeslope position of the catena the loess and colluvium were underlain by sand and gravel-sized sediments of similar mineralogical composition. These were thickly coated with a gel material. The advantage of the toeslope position is that translocation of the gel into an horizon that is essentially free of other clay-sized components has facilitated its isolation.

Fine ($<0.2\ \mu\text{m}$) and coarse ($0.2\text{--}2\ \mu\text{m}$) clay fractions of water-dispersed gel were separated by sedimentation and analysed by a range of instrumental and chemical techniques. In Table 1, and in Figs 1 and 2 the results are compared with data obtained from allophane isolated by water dispersion from the B and C horizons of a vitric Andosol, the Taupo sandy silt, from the Taupo Volcanic Zone of the central North Island¹⁰, and with that given by a synthetic aluminosilicate gel⁶ containing 60% by mass of Al_2O_3 .

Examination of the fine and coarse clay separates from the podsol by X-ray diffraction established their poorly ordered nature. X-ray diffraction patterns showed a broad hump in the baseline extending from 2.5 to 5.0 Å. The coarse clay fraction alone gave a weak diffraction peak at 3.35 Å which indicated the presence of a small amount of quartz. No evidence for the presence of cristobalite, feldspar, gibbsite, imogolite or phyllosilicates could be obtained from this technique or from thermal analysis, transmission electron microscopy or IR spectroscopy.

The IR spectra obtained from the podsol separates closely resemble that obtained from the synthetic gel (Fig. 1). Absorption maxima at 980, 550 and 350 cm^{-1} reflect a very low degree of silica polymerisation, and a corresponding dominance of Al—O , AlOOH and Al—O—Si bonds. The distinct shoulder at 1,080 cm^{-1} in the coarse clay spectrum indicates the presence of a silica-rich component in that fraction, but the absence of absorption at 800 cm^{-1} argues against the presence of appreciable amounts of discrete silica. The Taupo allophanes on the other hand have been shown to be mixtures of a highly aluminous gel phase similar to that found in the podsol or prepared synthetically, and a more siliceous comminuted volcanic glass phase¹⁰.

Molar $\text{SiO}_2/\text{Al}_2\text{O}_3$ ratios, based on total elemental composition, are lower for the podsol gels than for the Taupo allophanes (Table 1). As the latter are known to be mixtures of a gel phase and a comminuted glass phase, selective dissolution of the gel was attempted using both acid ammonium oxalate and sodium hydroxide as extractants^{11,12}. Both reagents extracted an alu-

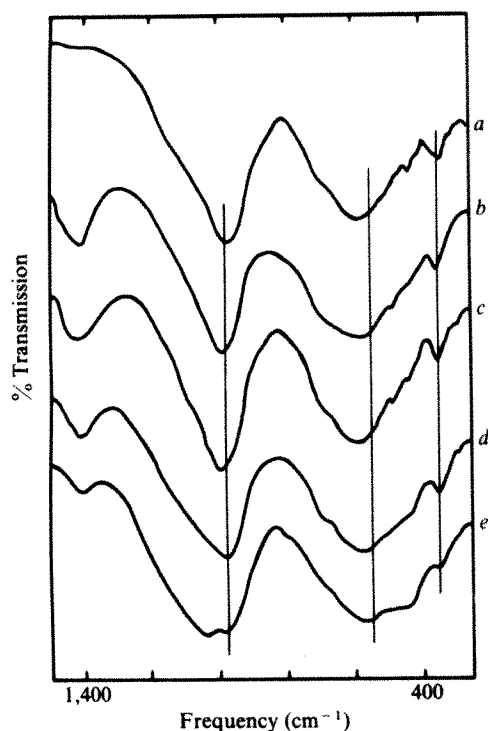


Fig. 1 Comparison of IR absorption spectra. Synthetic gel, *a*; podsol separates, *b* (fine clay) and *c* (coarse clay); and fine clay separated from Taupo sandy silt, *d* (B horizon) and *e* (C horizon).

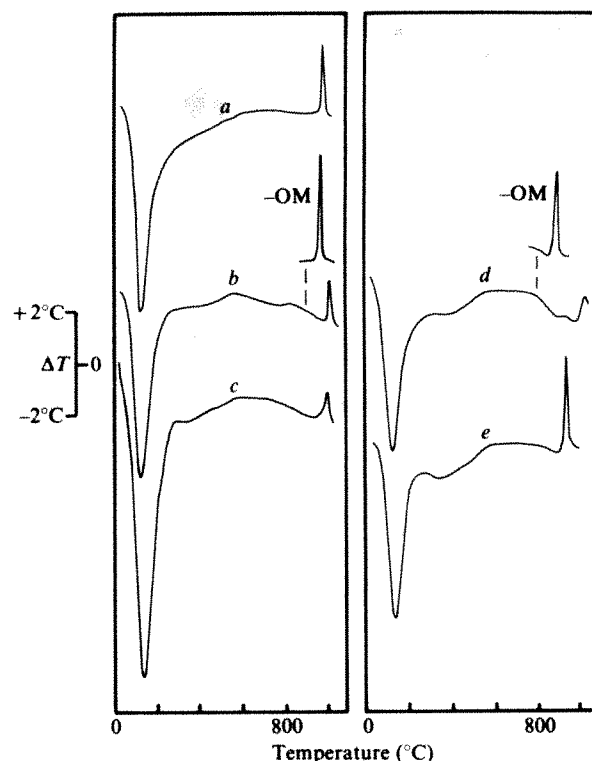


Fig. 2 Comparison of DTA thermograms. Identification of samples as for Fig. 1. Experimental conditions: sample masses, 50 mg (podsol), 80 mg (remainder); nitrogen atmosphere; -OM indicates that natural organic matter was removed by burning, 10 mg charcoal added, and sample reheated in nitrogen atmosphere.

minium-rich component. Unpublished data obtained in this laboratory have shown oxalate to be more specific than sodium hydroxide as an extractant of poorly ordered aluminosilicate gels in the appropriate experimental conditions. The $\text{SiO}_2/\text{Al}_2\text{O}_3$ mole ratios of the oxalate-extracted phases from both the podsol and Taupo samples are about half of the value of 1.0 commonly cited for allophanes¹³. Note that the reactive gels from the Taupo clays are more aluminous than those from the podsol, but hydroxyl release by fluoride⁵ indicates that the podsol contains the greater amount of allophanic material. The considerably higher $\text{SiO}_2/\text{Al}_2\text{O}_3$ mole ratios of the material extracted by sodium hydroxide from the podsol coarse clay and both Taupo clays indicates that this reagent has also partially dissolved the more siliceous phases known to be present in these samples.

The thermal characteristics of the fine and coarse clay from the podsol are almost identical to those of the Taupo allophanes and the synthetic gel (Fig. 2). All meet the defined requirements for allophane¹⁴. In addition, the effects of particle size, organic matter and carbon on the high temperature exotherm given by the podsol gels are consistent with those shown by allophane^{10,15}.

Fieldes has postulated that co-precipitation of weathering products in the subsoil of podsoles could lead to the formation of allophane¹⁶. Clearly, from the data presented here, the poorly ordered gel isolated from a podsol meets the widely accepted criteria for allophane. We have therefore confirmed that the genesis and persistence of allophane in podsoles is possible, and that its genesis does not require the presence of a poorly ordered parent material.

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Gyromagnetism and the remanence acquired by a rotating rock in an alternating field

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Although alternating field (a.f.) demagnetisation is routinely used in palaeomagnetism it has relatively recently been recognised that rotation of rock samples in the presence of an a.f. may introduce unwanted components of magnetisation. Doell and Cox¹ observed such magnetisations in 1967 but the phenomenon was not attributed to the effect of rotation until Wilson and Lomax² performed experiments about a single rotation axis and demonstrated the remarkable result that when an alternating field was applied at right angles to the axis of slow rotation, a remanent magnetisation was left antiparallel to the rotation vector after the a.f. had been smoothly reduced to zero. This magnetisation has been termed rotational remanent magnetisation (RRM). One of the explanations considered in attempts to account for the effect is that it is gyroscopic in origin, but order of magnitude calculations based on the rotation rate of the rock have indicated that such effects are insignificantly small^{2,3}, especially as RRM is detectable at rotation rates as low as 0.02 revolutions per second (r.p.s.). It is reported here that if appropriate assumptions are made regarding the magnetic behaviour of the rock, gyroscopic effects may be large enough to explain RRM.

When a rock is rotated at an angular frequency ω in a zero field, any magnetic moments within it will incline towards the rotation axis and a magnetic moment will appear antiparallel to the rotation vector. This is known as the Barnett effect and is a consequence of the intrinsic gyroscopic properties of the uncompensated electron spins which give rise to the moment of each magnetic particle. If the rock is static, the equivalent axial field B required to produce the same inclination of the moments towards the rotation axis is given by $\omega m/e$ where e/m is the ratio of charge to mass for the electron and where the magnetic properties of the particles within the rock are assumed to be due to electron spin rather than orbital motion. This equivalent field acts antiparallel to the rotation vector and is of magnitude $f_r \times 3.6 \times 10^{-11}$ T where f_r is the rotation frequency in revolutions per second (r.p.s.). For rotation rates of even several hundred r.p.s. it is thus insignificant compared to the fields required to produce a measurable remanence.

When, however, the rock sample is rotated in an alternating field, the changes in magnetisation which the rock experiences may be illustrated by Fig. 1, where for simplicity of explanation the alternating field has been represented by a series of moderately sharp pulses of alternate sign. Instead of considering

an anticlockwise rock rotation, Fig. 1 has been drawn with the rock stationary and the field axis rotating clockwise around it. A simplified explanation of the magnetisation changes in Fig. 1b are as follows. During the first pulse an isothermal remanent magnetisation (IRM) is acquired by the rock in position 1. Because the field axis is slowly rotating, the second pulse arrives at position 2 and as it builds up to its maximum value, it gradually removes the IRM acquired in position 1, modifying it by first flipping the low coercivity part anticlockwise towards the new direction but still exerting a torque on the high coercivity part which remains. This too is finally removed when the field reaches its maximum. During this build up of the second pulse, a positive (accelerating) torque (that is, in the same sense as the actual rock rotation) acts on the rock in Fig. 1b, c and d which are drawn for the rotation frequency (ω) less than the a.f. frequency (Ω). In Fig. 1e, however, where ω exceeds Ω , the torque is negative (decelerating) and the flip of the magnetisation is clockwise. This process is repeated in succeeding cycles.

During each flip, the rotation rate of the magnetic moments within the rock might be expected to be of the order of the reciprocal of the switching time or time constant (τ_t) of the flip. This may well depend on the coercivity of the particle but might be expected from a consideration of spin dynamics⁴ to be of the order of less than 10^{-8} – 10^{-7} s that is, the rotation rate of the magnetic moments during the flip will be of the order of 3×10^7 r.p.s. or greater. If the rock is stationary, as many moments will flip clockwise as anticlockwise and the system has perfect symmetry. Once rotation occurs, however, an asymmetry in the numbers of moments flipping in a particular sense is introduced and a corresponding torque will also be present as described above. The effective axial field B_r , which can be considered to deflect each moment towards the rotation axis as it flips towards the field direction, will thus be at least some 3×10^7 times greater than the value of 3.6×10^{-11} T quoted above, that is, about 1 mT or greater. This effective field is not a real field any more than is the anisotropy field, for example, but like the latter it is a useful concept. It is of the order of $\pi m/e\tau_t$ and will act in a direction antiparallel to the rotation vector describing the flip. For $\omega < \Omega$

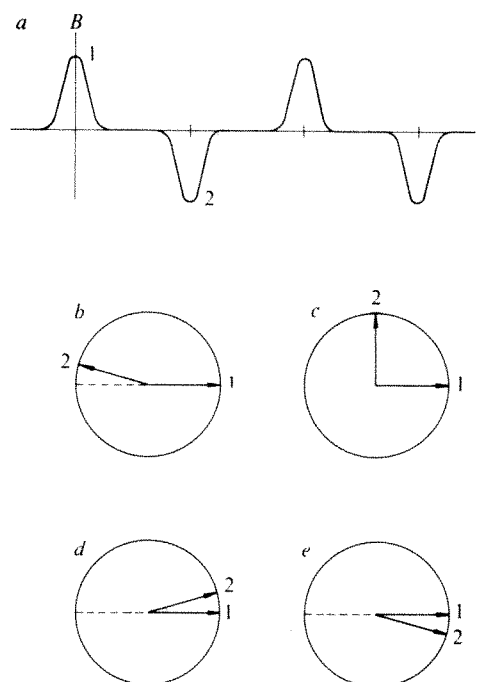


Fig. 1 a, a.f., for simplicity represented by a series of pulses. b, Direction of the first two field pulses as the field axis rotates clockwise around the cylindrical rock sample at a low rotation frequency (ω): c, when ω is half the a.f. frequency (Ω); d, when ω is just less than Ω ; e, when ω is just greater than Ω .

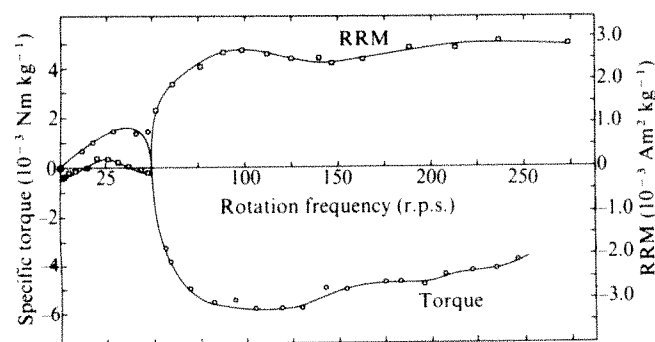


Fig. 2 The dependence of torque and RRM on rotation frequency for a peak a.f. of 51 mT for igneous sample WD34.

the flip is anticlockwise in Fig. 1, that is, in the same sense as the actual rotation of the rock, and thus the effective field is antiparallel to the rotation vector (that is, negative). When $\omega > \Omega$, however, the flip is clockwise and the effective field is thus positive. A transient effective axial field of the order of 1 mT acting just when the particle moments are flipping might be expected to produce a similar axial magnetisation to a constant field of 1 mT acting in the presence of the same transverse a.f. with no rock rotation if the sense of the flip were the same for all moments.

This discussion is clearly greatly simplified as the motion of each moment during the irreversible flip will presumably be that of damped precession⁵ under the influence of the external field and the anisotropy field, the latter reversing sign when the moment crosses the energy barrier separating the two equilibrium positions (for uniaxial anisotropy). To avoid an analysis of this complex motion the approach used above is an attempt to simplify the problem by considering the overall bias which is likely to be imparted to the motion of the particle moments (as a result of gyroscopic effects) as they are forced to flip by the external field. This bias is then interpreted as being due to an equivalent field B_e and the remanence which results from the action of this equivalent field is considered to be similar to an anhysteretic remanent magnetisation (ARM) acquired in a constant field in the presence of the much larger peak a.f. RRM is thus interpreted (like ARM) as being due to a statistical partial alignment of moments caused by a biasing field (B_e) in the presence of a much larger a.f. Because the moments do not all flip in the same sense, however, (the role of rotation is to introduce a bias favouring one particular sense of flip), the maximum RRM observed might be expected to be rather less than the ARM acquired in 1 mT in the conditions described above. The sign of RRM from this analysis is in agreement with the results of Stephenson³ who investigated RRM up to rotation speeds of 60 r.p.s.

To investigate RRM at higher rotation speeds, measurements were made on two igneous samples which were rotated at frequencies between 1.5 and 275 r.p.s. in a peak alternating field of 51 mT (ref. 5). This range of speeds was achieved by using a turbine driven from a compressed-air supply, with the rock sample mounted in the rotor. The magnetic torque exerted by the alternating field was measured by observing the initial rate of change of angular momentum imparted to the sample plus rotor when the field was suddenly applied to the stably spinning rotor⁶. The RRM was given to the sample by spinning the sample at the desired speed, applying the a.f. by quickly turning up the current in a surrounding coil by means of a Variac, and then after a second or two, smoothly reducing the current to zero over a few seconds.

Figure 2 shows the RRM and torque results for one of the samples coded WD34. In accordance with the above explanation, RRM is negative (except between 12 and 40 r.p.s.) for $\omega < \Omega$, and positive for $\omega > \Omega$. The fact that neither RRM nor torque exhibit further sign changes at higher rotation rates,

which might be expected from the simple explanation, because the a.f. is not, in fact, a series of pulses acquired during the decreasing part of the cycle is over a range of angles so that the torque is then always (retarding). A two dimensional numerical model based on single domain particles and a coercivity determined from the acquisition of IRM as a function of applied field has enabled a torque curve to be computed which is very similar to the above experimental result⁵.

The above description is greatly simplified, and, while the numerical model is more sophisticated than this, it is by no means a complete magnetic description of the rock, and therefore the unexplained experimental observation of the secondary sign change between 12 and 40 r.p.s. is possibly due to deficiencies in the model rather than because the above explanation is necessarily at fault. This secondary sign change was not so marked in the other sample and did not occur in the two samples investigated by Stephenson³.

The direct field required to produce an ARM of the same magnitude as the maximum RRM observed was about 100 and 20 μ T for the two samples (in a peak field of 136 mT rather than 51 mT). The estimated 1 mT transient effective field, which is thus a consequence of the sudden flip of the moments, which occurs when the field strength and direction becomes favourable would thus seem to be easily strong enough to account for the phenomenon of RRM. Moreover, this mechanism does not depend on the absolute rotation rate of the rock but only on the rotation of the field relative to the rock. This is in agreement with experimental results of Stephenson who found that it is immaterial whether the rock rotates or whether the a.f. axis rotates about the stationary sample³.

Perhaps RRM may thus be an example of a new type of remanence for which the name 'gyroremanent magnetisation' or GRM might seem to be appropriate.

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A gyroremanent magnetisation in anisotropic magnetic material

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An effect whereby an anisotropic magnetic material acquires a remanence on exposure to an alternating magnetic field (a.f.) is predicted from an analysis of the phenomenon of rotational remanent magnetisation (RRM). The remanence, which is of gyromagnetic origin, is produced even when there is no relative rotation between sample and field and all constant fields have been eliminated. The effect is demonstrated in magnetic recording tape and, as well as having important implications for a.f. demagnetisation techniques, may be relevant to magnetic recording processes.

The phenomenon of gyromagnetism is well known (see ref. 1 for a review) but it has not generally been considered that it could ever produce an easily measurable remanent magnetisation. It has recently been suggested, however^{2,3}, that the RRM identified by Wilson and Lomax in rock samples⁴, can be explained in terms of a gyromagnetic effect associated with a predominant sense of flip of the moments inside the sample as it

located in an alternating field. In a simplified approach to the complex problem of determining the motion of the particle moments during the flips, it has been suggested that, because of the sudden enforced rapid rotation of their associated angular momentum vectors, the particle moments can be considered to be deflected towards the rotation axis by a transient field B_g of the order of $\pi m_e / e \tau_f$ (where e/m_e is the charge to mass ratio for the electron and τ_f is the time of flip of the moment). It is this effective transient axial field, acting during the flip, which produces the remanence, and therefore in any system where a particular sense of flip predominates, such a magnetisation should result. Thus a rotating field of constant amplitude should also produce a remanence antiparallel to the field rotation vector, provided that the field is strong enough to produce the irreversible flips of the moments. In such a case, the resultant magnetisation should be independent of the rotation frequency f , provided that $1/f \gg \tau_f$. Of even greater interest, however, is that this approach leads to the prediction that rotation, either of the magnetic material or the magnetic field, is not a necessary condition for gyromagnetic effects to produce a remanence. This remanence, here termed a gyromagnetic remanent magnetisation (GRM), is likely to be produced whenever there is an asymmetry in the number of moments which flip in a particular sense.

Consider a uniaxial single domain particle orientated at an angle θ to an alternating field (see Fig. 1). Successive pulses of a weak a.f. merely cause reversible excursions of the moment away from the easy axis but a stronger field will cause the moment m to flip between the two easy directions (actually between two positions which are governed by the anisotropy field and θ). If θ is positive as in Fig. 1a, the sense of the flip will always be clockwise. That is, the rotation vector describing the flip is in the negative z direction. If, however, θ is negative as in

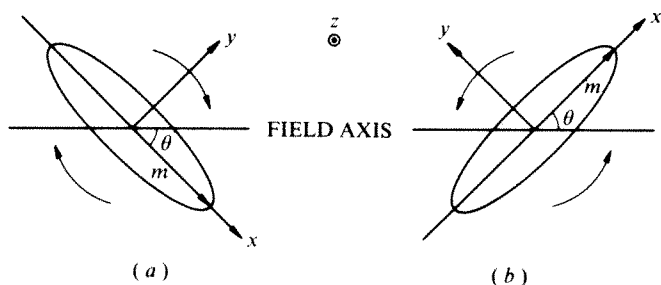


Fig. 1 A diagram to show the sense of the irreversible flip of the moment of a single domain uniaxial particle when subjected to an alternating field with peak value comparable to the anisotropy field.

Fig. 1b, the flip will be anticlockwise. As a result of the flip and because of the intrinsic gyroscopic behaviour of the moment of the particle, the moment will be deflected towards the $+z$ direction in Fig. 1a ($-z$ in Fig. 1b)^{2,3}. Clearly in the case under consideration, no remanence can be retained antiparallel to the flip vector because the moment will always lie along one of the two easy directions when the field is removed. In an assembly of particles, however, such as in a rock, where each particle has three unequal orthogonal axes representing its anisotropy, and where there are many different particle orientations, any inherent bias imparted to the overall motion of the moments of the assembly during the flips would be expected to produce statistically a remanent magnetisation in the opposite direction to the average flip vector in an exactly analogous way to that discussed previously in connection with RRM^{2,3}. In an anisotropic rock, therefore, with its easy magnetisation axis orientated along the x axis in Fig. 1 (making use of the same diagram), the bulk anisotropy will cause the isothermal remanence acquired in each pulse of the a.f. to be deflected towards the nearest easy direction, so that the same sense of flip as illustrated will predominate and an effective field antiparallel to the average flip vector will be present.

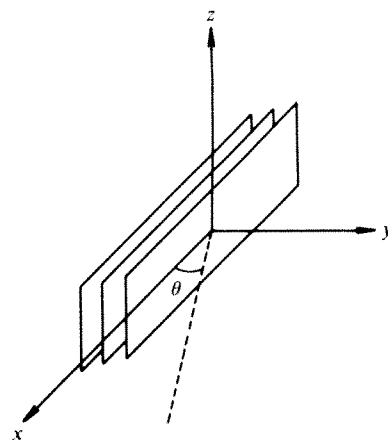


Fig. 2 Orientation of 100 layers of magnetic recording tape. The dashed line in the x - y plan indicates the a.f. axis for positive θ as in Fig. 1a.

This effective field will thus produce a GRM perpendicular to the a.f. and anisotropy axes. The direction (but not the magnitude) of the GRM will be given by the vector $\mathbf{A} \wedge \mathbf{F}$ where \mathbf{A} is a vector along the anisotropy axis. The field axis is specified by a vector \mathbf{F} , the direction of which is such that θ (the angle between \mathbf{A} and \mathbf{F}) always lies between $-\pi/2$ and $+\pi/2$. As the orientation of the field axis is thus altered through $\theta = 0$, the sign of the GRM should thus change, being negative (along the $-z$ direction) for $\theta < 0$, and positive for $\theta > 0$. The magnitude of the GRM might be expected to depend on θ , the flip time (τ_f) of the particle moments, and the magnitude of the anisotropy. For a very anisotropic sample, GRM might be expected to be of similar magnitude to the ARM acquired in a transverse a.f. of the same strength as that used to produce the GRM, and in the presence of a steady field of magnitude $\pi m_e / e \tau_f$.

To test this hypothesis an anisotropic sample was fabricated by stacking together 100 strips of magnetic recording tape (see Fig. 2). Each strip was of dimensions 18 mm \times 6 mm. The long axis of each strip (x axis) was mounted horizontally in a non-magnetic holder with the plane of each strip vertical (z axis).

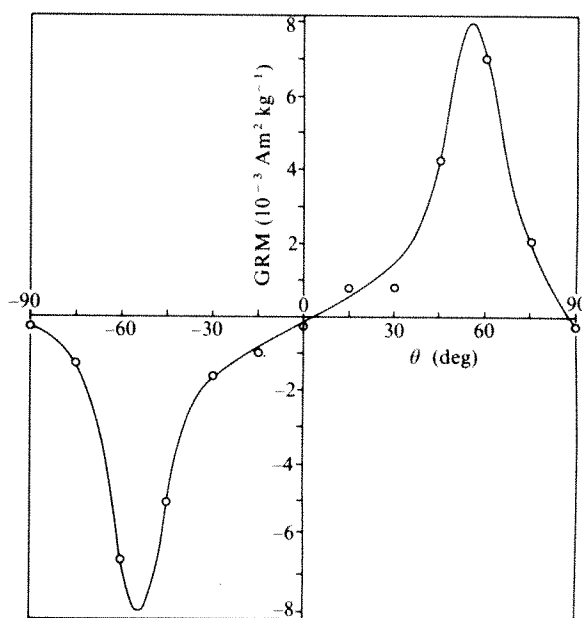


Fig. 3 The dependence of the z axis magnetisation (which is a GRM) for 100 layers of magnetic tape when subjected to a 274 Hz alternating field of 50 mT peak at an angle θ as in Fig. 2.

Initial susceptibility measurements yielded values of 128 and $85 \times 10^{-6} \text{ m}^3 \text{ kg}^{-1}$ respectively (per unit mass of tape), in directions along and perpendicular to the plane of the strips. Because the distribution of the particles is essentially isotropic in the x - y plane⁵ these differences in susceptibility are due to the differences in demagnetisation factors in the two directions. Nevertheless this shape anisotropy should produce the same sense of flip as illustrated in Fig. 1 when the x direction coincides with the long axis of the tape.

The tape was demagnetised by tumbling the composite sample in an a.f. demagnetiser which reduced the remanence to a suitably low value. This was measured on a spinner fluxgate magnetometer used for measuring the remanence of rock samples. The sample was then orientated at various angles as in Fig. 2 inside a set of Helmholtz coils which cancelled the Earth's field. The a.f. was increased to a peak value of 50 mT (at 274 Hz) and then slowly decreased over a time of about 1 min and any resulting remanence was measured. Between each different orientation the sample was demagnetised by tumbling. A z -axis remanence did indeed result from this procedure and this is plotted as a function of θ in Fig. 3, where it is apparent that the GRM has the general form expected from the preceding analysis. This result should be independent of a.f. frequency f , provided that $1/f \gg \tau_r$.

When the pair of Helmholtz coils cancelling the Earth's vertical component was switched off and a field of $44 \mu\text{T}$ acted along the $-z$ direction, the magnetisation changed from 7 to $-21 \times 10^{-3} \text{ Am}^2 \text{ kg}^{-1}$ at $\theta = +60^\circ$. This suggests that an ARM of about $28 \times 10^{-3} \text{ Am}^2 \text{ kg}^{-1}$ is being added in opposition to the

GRM, so that the effective field producing the GRM, at least when the peak a.f. is 50 mT, is equivalent to a steady ARM field of about $11 \mu\text{T}$. This value is not unlike those estimated from RRM experiments on rocks^{2,3}.

The implications of this result are considerable for the a.f. demagnetisation of anisotropic rocks, since it implies that a single axis non-tumbling demagnetisation system will invariably introduce a GRM into the sample unless the anisotropy axes of each rock happen to be orientated at 0 or 90° to the a.f. axis. Such magnetisations in the past may well have been misinterpreted as ARM, but an orientation dependence check should indicate the presence of a GRM. Such a dependence is now under investigation in a rock sample and will be described at a later date. This effect may also turn out to be of importance in magnetic recording. It may also prove to be a useful tool in studying magnetisation rotation process in magnetic materials and anisotropy in rocks and minerals.

Of perhaps even greater interest, however, is that gyromagnetism can produce an easily observed remanence in magnetic materials even when they are merely exposed to an alternating field, and this would appear to be an effect which has hitherto been unsuspected.

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A braidplain facies model for the Westphalian B Coal Measures of north-east England

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The Westphalian B (Coal Measure) sediments of Northumberland, Durham, and the other northern English coalfields were deposited on extensive coastal plains between several peripheral source areas and the Saint George's Land block to the south. We report here a facies model resulting from studies of fluvial sandstones in the Westphalian B of Northumberland and Durham and suggest that sandy braidplains were an important feature of the depositional environment in this coalfield. Braidplain sand sheets, and associated washouts in underlying coal seams, formed following tectonic uplift of local source areas supplying recycled sands. Parts of this model may be extended to other coalfields in northern Britain.

It has often been assumed that the Coal Measures environment was a large-scale delta top^{1,2}, with peat-forming swamps laterally equivalent to meandering rivers, and a marine influence becoming more important towards the central Pennine coalfields³. Detailed interpretations of parts of the Pennine coalfields have been made as the deposits of swamp, lake, lake delta and river channel environments^{4–6}, but no model to explain the major sedimentary features of this coalfield, or the differences between the different Pennine coalfields, has emerged.

In the Northumberland and Durham coalfield the Coal Measures can be divided into three main sedimentary facies associations. (1) The marine band facies consists of distinctive fossiliferous silty mudstones up to 2 m thick. These are interpreted as the deposits of occasional, low energy marine transgressions. (2) The coal-bearing facies consists of part or all of a sequence which, when complete, comprises black fossiliferous

shales, passing up into grey, finely laminated mudstones and siltstones and overlain by seat-earth and coal. These siltstones may also contain very fine-grained sandstones, forming sheets 10–50 cm thick, of limited lateral extent, and often containing bioturbation, climbing ripple or trough cross-lamination structures. This is the dominant facies association; it ranges from 10 cm to 15 m in thickness and is interpreted as the deposits of shallow lakes with small delta infills, capped by swamp vegetation. (3) The major fluvial facies comprises fine to very coarse sandstones 4–35 m thick, with no autochthonous coal. Each sandstone has an erosional base and fills any washouts in the underlying coal seam, so any understanding of these major sandstones and their associated washouts is of great economic importance to coal exploitation. The lower part of the sandstone is often conglomeratic, containing locally eroded clasts of ironstones and angular partially lithified silts, together with clasts of coalified once-leathery peats and fossilised log jams. The sequence passes up into trough and tabular cross-bedded sandstone in sets 1–3 m thick forming cosets 5–15 m thick with unimodal palaeocurrent vectors paralleling washout trends. These are followed by about 2 m of ripple-laminated siltstones and fine sandstones, and may be capped by seat-earth and coal of the coal facies. There is no evidence for any significant systematic lateral river migration. This type of sequence is interpreted as the deposits of a river in which a rapid, unidirectional flow decelerated, allowing silting of the channel and eventual blanketing by vegetation. Field and borehole data show that these sandstones form thin sheets, usually 10–15 m thick, but occasionally up to 35 m in thickness. These can cover large areas of the coalfield ($>1,500 \text{ km}^2$), but are not continuous, leaving occasional 'islands' of older sediments. These major stratigraphic and facies relations are quite unlike those of migrating and avulsing fluvial systems⁷ and we suggest that the vertical sequences are not due to the migration of laterally equivalent facies, but rather to major environmental changes with time. These sandstone sheets probably resulted from the periodic invasion of high-energy braided rivers eroding into and spreading over an otherwise low-energy coastal plain, to form large areas of sandy braidplain (Fig. 1). There were no major deltas. This implies periodic rejuvenation of the river system by changes in discharge or of slope, possibly originating from

tectonic, climatic or eustatic changes. Thus in any one seam, washouts were formed synchronously after river rejuvenation when the stream power was rapidly increased, but before erosion produced a significant sediment load. Consequently, many washouts are narrow, deep and of low sinuosity, but as the rivers lost power and aggraded vertically, they continued to widen, often by eroding along the tops of peat seams which formed underlying cohesive layers (Fig. 2). Because these major sandstone events were time horizons, they may be of value for seam correlation.

In Northumberland and Durham several braidplain sandstones fill washouts trending east/west, whereas only a few fill north/south washout trends. Palaeocurrents deduced from major bedforms and coincident washout trends may be directed eastwards or northeastwards in one sandstone, and be succeeded by westward-directed palaeocurrents and washouts in the next. This periodic palaeoslope reversal implies tectonic tilting from sandstone to sandstone. The palaeoslope reversal is also reflected in a difference in lithology between successive sandstones, suggesting different source areas. For example, a sub-rounded/rounded subarkose with orthoclase and minor muscovite may be succeeded by a sub-rounded subarkose with perthitic and microcline feldspars, and minor garnet. All of these features can best be explained by repeated tectonic uplift and erosion of local sedimentary source areas to the west, north and east, rather than by a single large river system depositing far-travelled detritus. Note that this province was probably tectonically active during the Westphalian^{13,14}. Thus it is possible that much of this mature, rounded, sandy detritus was reworked from easily eroded areas of Namurian or older sediments on the Southern Uplands, Mid North Sea High and North Pennines. Such source areas only a few tens of kilometres distant need only have been uplifted by a few tens of metres to have triggered off erosion and provided a slope sufficient to maintain braiding in sandy sediment, given a reasonable discharge variation^{10,15,16}. Away from the source areas river slope decreased. This caused a decrease in river power, deposition of the coarser

bedload, and a transition to meandering rivers further southwards (Fig. 1). After the source areas had been reduced by gullying, removing any stored sediment¹⁰, the braidplain would silt up, and the coal facies re-establish. Occasionally, some of the large braidplain events influenced the distal central Pennine areas, causing unusually widespread washouts and sands. The silts and very fine sands normally deposited on this coastal plain show evidence of river migrations and avulsions^{4,5}, however, these features were only well developed in the distal parts of the overall coastal plain.

Climatic change is unlikely as a cause of this river rejuvenation, as such a change would have had to have been repeated rapidly many times within the Westphalian B and would not explain such divergent palaeocurrents and source areas.

Worldwide eustatic sea-level changes are not favoured as the cause of river rejuvenation because of the palaeocurrent, petrographic and proximal to distal features mentioned above. During eustatically induced regressions^{8,9}, any fluvial transportation would be expected to reach across former shelf or coastal plain areas, and deposit sands in shoreline areas^{8,10,11}. While each regression continued, erosive gaps and a recurrent linear washout trend down the palaeoslope would be expected to have developed in these coastal shelf areas. However, the evidence above, together with borehole information from Northumberland and Durham, does not support this in the Westphalian B.

The idea that the Northumberland, Durham and probably Cumbrian coalfields were slightly elevated proximal areas with respect to the other Pennine coalfields is supported by regional marine band variations. These proximal coalfields have thinner marine bands containing a low diversity near-shoreline fauna and several bands were not deposited at all³. This suggests that the sea was unable to transgress into these proximal areas as often, or for as long a period, as elsewhere. The Mid-Westphalian A to Mid-B has very few marine bands and contains the most productive coal seams¹⁷. The prevailing regional subsidence may have been matched by clastic supply¹² during this

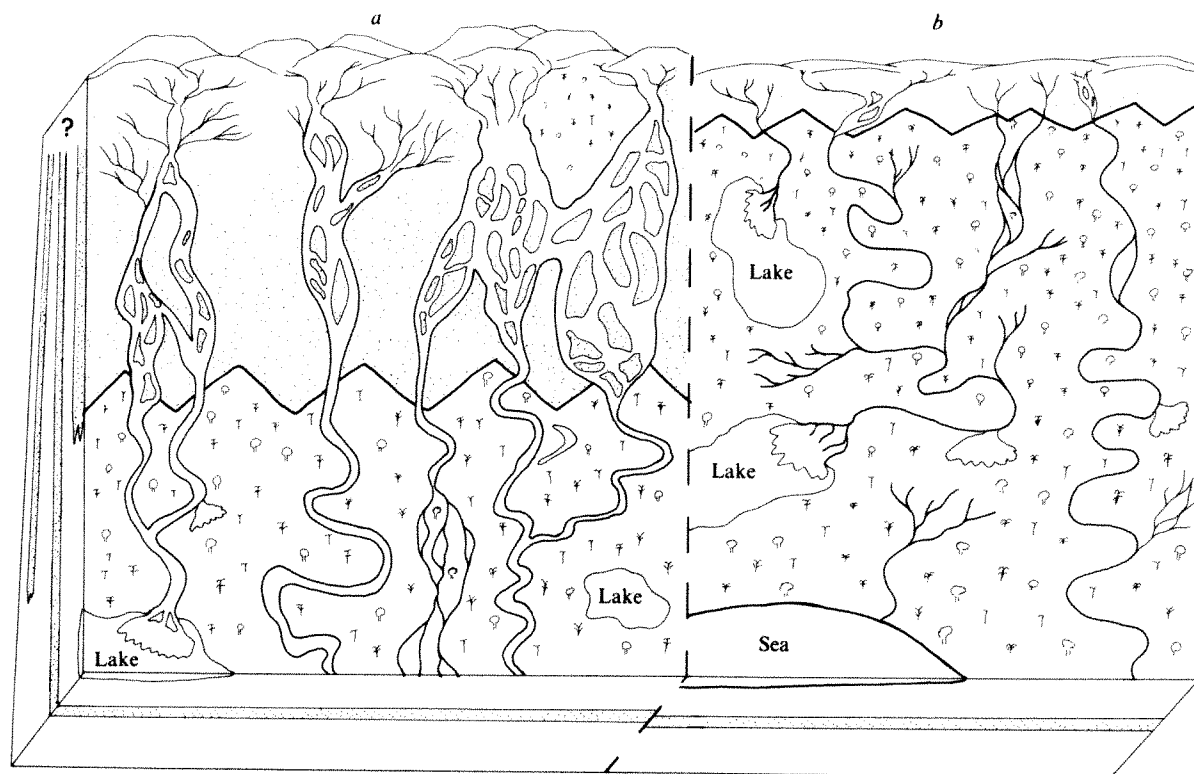


Fig. 1 Schematic palaeogeographical block diagram showing large-scale environmental changes in Coal Measures. Not drawn to scale, but a distance of several hundreds of kilometres from source areas to sea is envisaged. Plan shows proximal to distal changes proposed for: *a*, a period of maximum braidplain development, passing into coastal plain swamp. Source areas were uplifted. *b*, The coastal plain swamp encroached as the braidplain was abandoned. Source areas now eroded. Block section shows the extent of previous braidplains, but not detail of coal or marine facies.

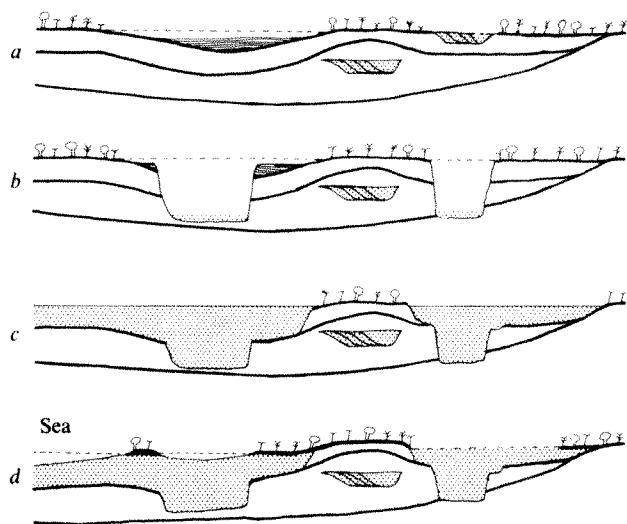


Fig. 2 A schematic cross-section perpendicular to palaeocurrent flow shows the sequence of events resulting from river rejuvenation. Horizontal scale greatly shortened. *a*, Coastal plain coal facies, fed by small low energy avulsing and meandering rivers, which deposited predominantly fine-grained sediments and formed minor lake deltas within a swamp environment. *b*, Uplift of source area by only 10–20 m, not necessarily synchronous or of equal amount in all source areas, caused river rejuvenation. Rivers formed new channels along lines of least resistance through topographically low areas such as pre-existing rivers, or old lake basins. These rivers initially carried little coarse sediment and rapidly cut down into the older coastal plain sediments, forming low sinuosity washouts where peat was eroded. *c*, In areas proximal to source, the rivers quickly received coarse sediments and aggraded. They then rapidly widened along the top of the cohesive peat layers by bank collapse, forming basal conglomerates. Sand load continued to be deposited in proximal areas forming a braidplain, and usually only the fine sediments were carried down to distal coalfield areas, where washouts from the initial increased water discharge were filled with silts and fine sands. *d*, As the source area became rapidly eroded and aggradation continued, a near equilibrium base level was restored and rivers lost their power and silted up to form large shallow lakes. The distal coastal plain coal facies gradually encroached northwards and swamp conditions resumed with steady state graded rivers. If regional subsidence was too rapid, the sea transgressed, forming a marine band.

period. Conversely, in the Upper B, regional subsidence often outpaced supply, resulting in many marine transgressions. It seems likely that such transgressions had a significant tectonic and sedimentary control independent of eustatic sea-level changes. The large time range estimated for a goniatite zone fauna⁹ may be significant in the correlation of some apparently more widespread marine bands.

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Silurian or Upper Ordovician fossils at Guolasjav'ri Troms, Norway

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In August 1979, specimens of rugose and tabulate corals, crinoids and brachiopods (Fig. 1*a*) were recovered from metalimestones in a belt extending 0–3.5 km north-west of Guolasjav'ri, a mountain lake south-east of Kåfjord, Troms, in Norway (Fig. 2, locality I). This is the first fauna to be recognised in this part of the Caledonian nappe sequence in Troms. The presence of corals proves that these metalimestones are not older than Middle Ordovician which imposes constraints on the correlation of this series with units in the Caledonides to the north and south and has an important bearing on the problematic relationship between the dominant Scandian phase (mid-Silurian¹) of the Central Scandinavian Caledonides and the Finnmarkian phase (late Cambrian²) of northernmost Scandinavia.

Padgett³ erected the first lithostratigraphy in this part of the Norwegian Caledonides (Fig. 3). Within his 2,600 m non-granitised Birtavarre Series, he named the unit in which the fauna has been found the Guolas Limestone Series. Where the fossils are preserved, this has undergone Barrovian-type, amphibolite-facies regional metamorphism synchronous with polyphasal deformation (our unpublished data). These fossils are therefore poorly preserved, although they remain surprisingly little deformed. The two least altered specimens are identified as halysitids with ordinary lacunae and elliptical corallites about

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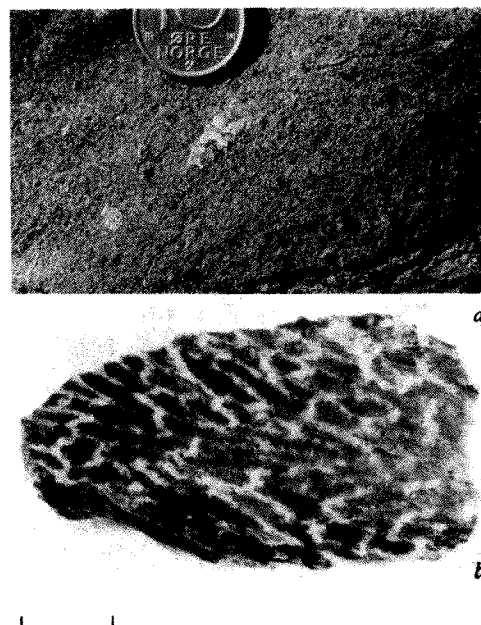


Fig. 1 Photographs of the fossils. *a*, Crinoid ossicles and a fragment of a ribbed brachiopod at outcrop locality 1 (Fig. 2). Coin is 1.5 cm in diameter. *b*, Halysitid tabulate coral, cut surface; from locality 2 (Fig. 2). Scale bar, 0.5 cm.

1 mm wide in cross-section (Fig. 1b), indicating a Silurian or possibly late Ordovician age.

Although tectonic dislocations (for example, the Capps Thrust) were recognised³ within the Kåfjord sequence, the stratigraphy was not thought to have been disrupted significantly. Another thrust^{4,5}, however, below the lower quartzite and marble units, not recognised by Padget, forms an important regional nappe boundary (see for example, ref. 6 and Fig. 3). Recent field work (largely by R.E.B. to be detailed elsewhere), also supported by evidence farther afield, has demonstrated a simpler stratigraphy than that of Padget and other workers^{7,8}. The succession (Fig. 3), including horizons of intermediate or acid lavas in a 350–400 m thick basic pillow-lava accumulation near locality I, appears to be repeated by large-scale, sub-recumbent folds. Way-up evidence (mainly from pillow shape and the clast content of an immediately overlying polymict conglomerate) supports a normal stratigraphic order in the fossiliferous part of the sequence just north of Guolasjav'ri.

One of the outstanding problems of the Kåfjord sequence has been its correlation within the Scandinavian Caledonides. Zwaan and Roberts⁸, although recognising repetitions within Padget's³ Kåfjord stratigraphy, which they sought to explain entirely in terms of thrusting, correlated these rocks, in accord with the orthodox view^{2,9–11}, with the upper portion of the well documented Sørøy stratigraphy¹² (Fig. 2). The late Precambrian–late Cambrian age of the latter is based on an early to middle Cambrian archaeocyathid fauna¹³ and on radiometric age dating (see refs 2, 14). Quenardel's⁷ interpretation seems to be largely a combination of those of Padget³ and Zwaan and Roberts⁸. In contrast, some economic geologists¹⁵ postulated an Ordovician age for the ore-bearing rocks within the Kåfjord sequence on account of the similarity in ore paragenesis between these sulphide ores and some farther south in Scandinavia.

The lithostratigraphic correlation between the Kåfjord and Sørøy sequences led Sturt, Roberts and others^{2,7,8,11,16} to assume without reservation that the late Cambrian Finnmarkian orogenic phase extended through the Kåfjord area and much farther south into Nordland and adjacent parts of Sweden. They thus discounted the previous correlations^{17–20} between the southern part of the area and fossiliferous Ordovician–Silurian (Köli) rocks a little to the south. The discovery of a late Ordovi-

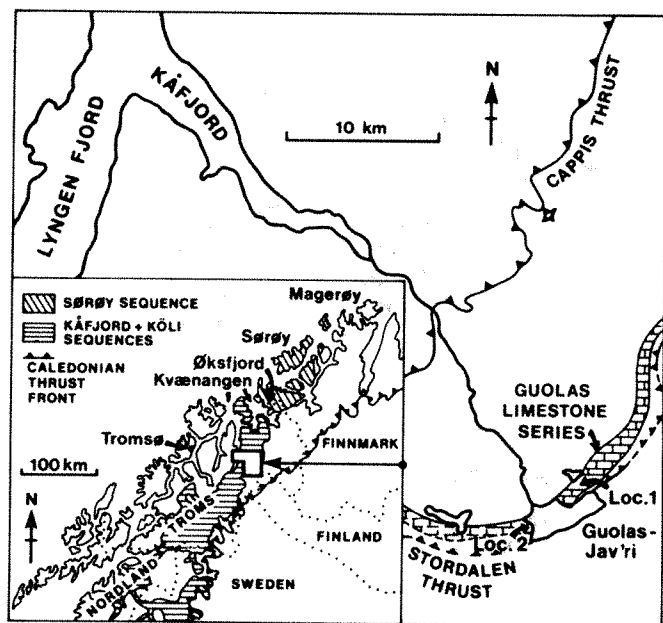


Fig. 2 Map of the Kåfjord region to show the fossiliferous localities in the outcrop of the Guolas Limestone Series. Inset shows location of the Kåfjord region within the outcrop of the Köli/Kåfjord/Sørøy sequences.

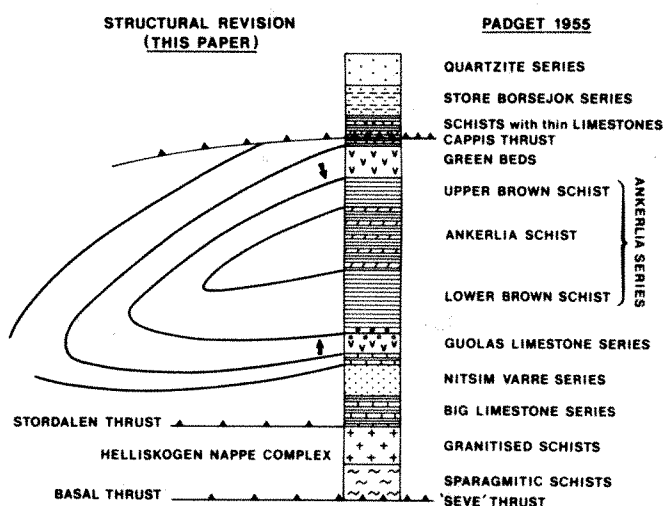


Fig. 3 The interpretation of the stratigraphy of the Kåfjord region according to Padget³ (right) and the revision proposed in this work (left). The column is not to scale—the sequence above the Stordalen Thrust (Padget's non-granitised Birtavarre Series) is ~2,600 m thick.

cian–Silurian fauna in the Kåfjord rocks which are shown to belong to a stratigraphic sequence only disturbed by tectonic duplications, makes the lithostratigraphic and tectonic correlations of this sequence with that of the Köli and Gasak nappes^{18,20} of central Nordland and adjacent parts of Sweden discussed by Binns⁶ more likely. Geochemical comparisons between the volcanic rocks of the Kåfjord and Köli sequences are under way and these may strengthen this correlation. Padget's Capps Thrust may define the nappe boundary but lithological and tectonic complications south-west of Kåfjord prevent this from being more than a tentative proposal at this stage.

Even an approximate chronostratigraphic correlation between the Kåfjord and Sørøy sequences can no longer be entertained. It seems possible to trace the Kåfjord sequence laterally through to the Kvaenangen–Øksfjord area^{3,4,8,9,23,24} (Fig. 2). At Øksfjord the strata are invaded by the syn-orogenic (Finnmarkian phase; climax 500–550 Myr ago²) Seiland Igneous Complex^{21,22}. If the lithostratigraphic correlation and the Sørøy region isotopic age evidence are to be relied on, it is difficult to explain the ensuing relationship without invoking rapid diachronous southward younging of the active orogenic belt and of regional sedimentation during late Precambrian–late Silurian time. Neither geophysical work²⁵, nor mapping^{9,21,24} have revealed a major transverse tectonic break between Øksfjord and Kvaenangen and tectonic interleaving of older and younger nappe units is incompatible with the correlation of Hooper and Gronow²³ and Armitage *et al.*⁹. Equally rapid tectono/thermal diachroneity has been recognised in Cenozoic triple plate junction migration²⁶ but sedimentation diachroneity of at least 100 Myr in a lateral distance of about 100–200 km (an estimate of pre-folding relationships) seems inconceivable. Hence we conclude that the correlations between Øksfjord and Kåfjord, and the Sørøy region isotopic age evidence require critical re-consideration. Perhaps the existence of an independent Finnmarkian Orogenic belt is in doubt.

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A new hominid fossil skull (L.H. 18) from the Ngaloba Beds, Laetoli, northern Tanzania

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In 1976, a fossil hominid skull was recovered from the Ngaloba Beds at Laetoli, Northern Tanzania; its morphology is discussed here. The discovery of this skull is of great interest and importance because of its very substantial presumed antiquity and its largely anatomically modern morphology. The discovery has considerable implications for the antiquity and origin of modern *Homo sapiens*, a subject of longstanding interest and one which has gained renewed attention recently.

The Ngaloba Beds¹, lying above the vogesite lavas which separate them from the underlying Ndolanya and Laetolil Beds^{2–4}, are stream deposits, principally sandstones and claystones, of which only patches are preserved. These patches consist chiefly of detritus eroded from the underlying Ndolanya and Laetolil Beds and they contain artefacts of Middle Stone Age affinity. The skull was recovered from a 2-m thickness of the Ngaloba Beds at Locality 2 that also yielded artefacts, some fossil reptilian and avian bones as well as fossil mammalian bones. This exposure is principally of sandy claystone and contains a water-worked vitric tuff. The tuff is trachytic and contains the pyroclastic minerals biotite and anorthoclase; it is tentatively correlated with the marker tuff in the lower unit of the Ndutu Beds at Olduvai Gorge⁵. This is the only trachytic tuff younger than Bed IV in Olduvai Gorge and its age is estimated at $120,000 \pm 30,000$ yr BP (R. L. Hay, personal communication). The skull was found by E. Kandini *in situ* but eroding out of the deposits.

The skull is almost complete and includes the bones of the vault, much of the base, both temporal bones, part of the

sphenoid and much of the face including the palate and part of the upper dentition. The bones are all heavily mineralised with no signs of pathology, but there are signs of post-mortem plastic deformation that has resulted in torsion to the right of the supraorbital region and some springing of the temporo-occipital suture on the left. As recovered, the skull was in 22 pieces and coated with greyish calcareous matrix. Cleaning produced fossil bone of an ivory colour and natural texture with the preservation of remarkable surface detail. It was possible to reassemble the vault and base into one structure and the paired maxillae into



Fig. 1 Left lateral view.

another. There is no point of contact between the facial skeleton and the calvaria although very little bone is missing. The relationship between these two main fragments of the skull remains speculative. The age at death seems to have been between 18 and 30 because the sutures of the vault are all open, but one third molar is present and fully in wear. The state of wear of this tooth suggests that the upper end of the age range is most likely.

Dimensions of the skull are given in Table 1. In lateral view (Fig. 1) the skull shows several striking features including marked recession of the forehead, a rounded occipital profile, an undercut central occipital torus and a small mastoid process. The frontal view (Fig. 2) shows a divided supraorbital torus, a relatively low vault and a mid-parietal swelling. The frontal bone is very slightly keeled in the sagittal plane but there are no

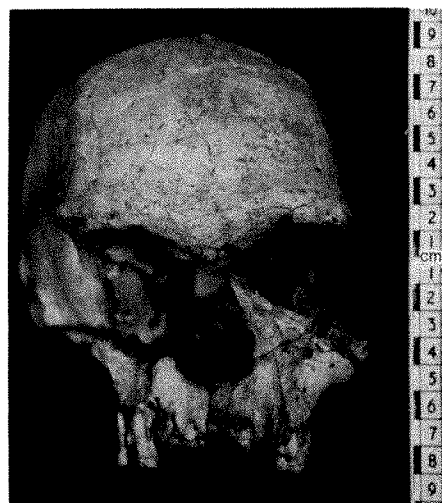


Fig. 2 Frontal view.

Table 1 Skull dimensions

Greatest length (glabella/opisthocranion)	—	205 mm
Greatest breadth (biparietal)	—	140 mm
Cranial index	—	68.3
Vault thickness (right and left parietomastoid and bregma)	—	12 mm



Fig. 3 Occipital view.

marked parasagittal flattenings. In occipital view (Fig. 3) the general frontal profile is confirmed but the parietal vault expansion is more clearly seen in the form of distinct parietal bossing or angulation.

The basal view (Fig. 4) of the skull shows plastic deformation which has affected the left frontal bone and to some extent the position of the left temporal bone. There are well marked frontal sinuses exposed by the loss of the ethmoids; the sinuses extend laterally into the orbital roof and posteriorly between the tables of the skull. The sphenoid is preserved in part and includes part of the body, greater wing and lesser wing of the right side and part of the body on the left. Both temporal bones are present and the right one is virtually complete. Both styloid processes are broken off but both the fossae for articulation of the mandible are present. The foramen magnum and its associated structures are entirely missing.

Both mastoid processes are limited by deeply incised mastoid notches (digastric grooves), and on the right there is a prominent occipitomastoid crest. The cranial capacity has been estimated by the water displacement of a cavity cast and the mean value of six estimates is 1,200 cm³ with a small range of observational error.

The facial fragment consists of the paired maxillary bones including the hard palate, the alveolar processes bearing some teeth as well as both zygomatic processes and the frontal process on the left. The dental arcade is U-shaped and the palate is deep



Fig. 4 Basal view.

and broad. The teeth that are present include P³, P⁴ and M¹ on the right, and the stump of P⁴ as well as M¹, M² and M³ on the left. The dimensions of the teeth are given in Table 2. All the teeth are heavily worn, with no trace of the cusp and fissure pattern on any tooth. Dentinal exposure is present on all the molars and all the teeth have suffered from post-mortem damage. The nasal aperture is pear-shaped and there is evidence of the presence of a nasal spine.

Table 2 Dimensions of the teeth*

		1 ¹	1 ²	C	P ³	P ⁴	M ¹	M ²	M ³
Left	B/L breadth	—	—	—	—	Roots only	11.5	—	10.5
	M/D length	—	—	—	—	—	10.0	—	10.0
Right	B/L breadth	—	—	—	9.5	9.8	12.5	—	—
	M/D length	—	—	—	7.0	7.0	10.8	—	—

*All the teeth are heavily worn and damaged.

In frontal view the zygomatic processes both take off laterally well above the alveolar margin and angle sharply laterally, giving breadth to the face. In lateral view the face shows a marked degree of subnasal prognathism so that the anterior dentition would have projected to a considerable degree. The frontal process of the maxilla on the left is broken, preventing articulation of the face with the cranial vault. The distortion of the frontal bone has also made correct positioning of the two major fragments impossible for all views of the skull.

The final assessment of the affinities of the Laetoli Hominid 18 skull must await a full study including anatomical comparisons and metrical analysis. From a preliminary examination, however, there is little doubt that it should be regarded as an early East African example of sub-Saharan *H. sapiens*, of particular interest because of its good state of preservation including the presence of its facial structure. The skull shows a number of points of resemblance with the African skulls known as Omo I, Omo II (ref. 6), Broken Hill (Kabwe), Saldanha, Bodo⁷ and Ndutu^{8,9}; its anatomical features are mixed in that it has some modern characters and some that are archaic. The general expansion of the vault, the rounded form of the occiput and the low position of the inion are modern features whereas the frontal flattening, the supraorbital torus, the small mastoids, the occipitomastoid crest and its general thickness are archaic features.

The dating of the skull also adds considerably to its importance as it places the fossil near to the root of the evolution of *H. sapiens* in East Africa out of the early *H. erectus* stock represented by Olduvai Hominid 9 and KNM-ER 3733. The *H. erectus*/*H. sapiens* transition is still poorly documented despite the recent finds from East Africa, notably the Bodo skull and the skull from Ndutu. The recovery of Laetoli Hominid 18 adds to this growing sample and makes an important contribution to our understanding of the evolution of *H. sapiens* in sub-Saharan Africa.

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Aggressive tusk use by the narwhal (*Monodon monoceros* L.)

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The narwhal, an Arctic odontocete, has two horizontally embedded teeth at birth¹. In males and a few females, the left tooth erupts at the end of the first year (K. A. Hay, personal communication) and develops into a spiralled tusk, which can be up to 260 cm long. It has been suggested that the tusk is used to disturb potential benthic prey²; to pierce prey before killing³; to pierce thin ice to make breathing holes^{4,5}; as a defensive weapon⁶; as a cooling mechanism⁶, and as a sound transmitter^{7,8}; but it is most probably used in display or fighting⁹⁻¹³. There is no mention in the literature of overt aggressive behaviour between narwhals or of scars on narwhals. We have found many scars on adult male narwhals. These, together with the high incidence of broken tusks in adult males and the burst of tusk growth at sexual maturity indicate that tusks are used in aggressive encounters.

Narwhal behaviour was observed from the ice and from a cliff on northern Baffin Island in the eastern Canadian Arctic during three field seasons, from June to October of 1976-1978. Narwhal specimens, which were provided by Inuit hunters, were sampled throughout the field seasons. We measured body length, tusk length, girth and weight, and counted from photographs the number of scars visible in a frontal view on the forehead and above the mouth of each sample.

Narwhals cross their tusks^{3,9}, and strike them against one another (R. Gray cited in ref. 14). We frequently observed narwhals cross tusks above and below the water surface (Fig. 1). Tusk crossing was often accompanied by a great deal of social behaviour, which is described by Silverman¹⁵. Agonistic behaviour, indicated by abrupt and quick movements, was rare. We propose that the observed behaviour patterns are components of mating and rutting behaviour sequences. Such behaviour, which was observed outside the mating season (mating is from March to May (ref. 16 and unpublished results of K. A. Hay)), may assist juveniles to develop skills for adult sexual roles, and may also communicate dominance rank.

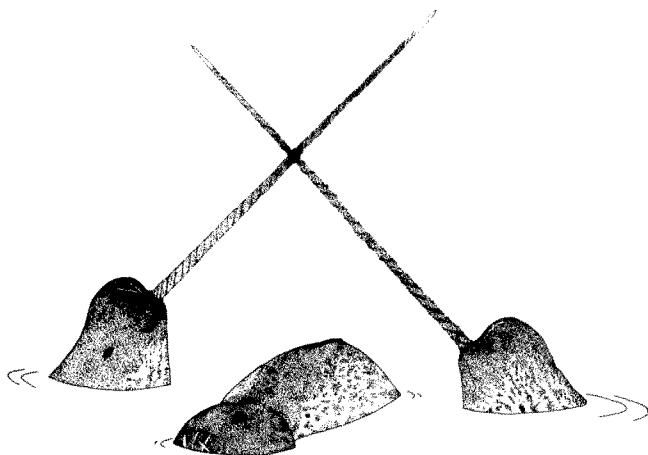


Fig. 1 Drawing of two male narwhals crossing tusks above the back of a third narwhal.

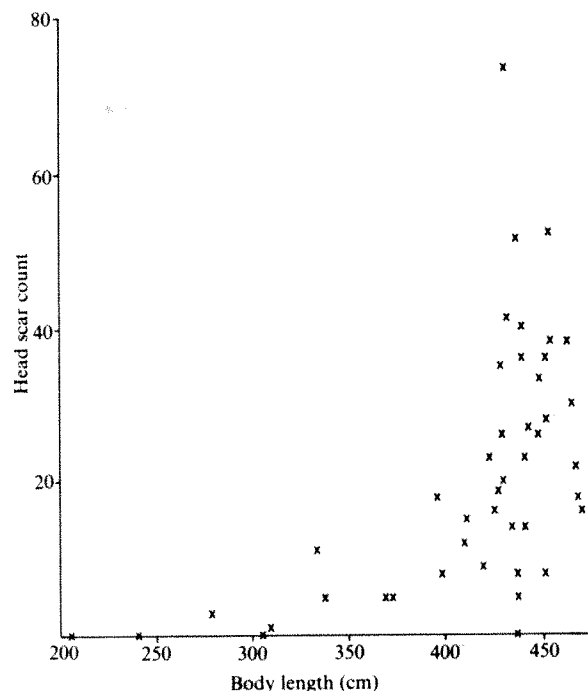


Fig. 2 Relation between body length and head scar count of male narwhals. Regression equation is: $y = 1.576 \times 10^{-12} x^{4.953}$; $r = 0.756$; y, head scar count; x, body length in cm; n, 44.

The mean number of scars on the heads of adult males was significantly greater than that on adult females ($P < 0.001$). Males become sexually mature at body lengths of about 400 cm (K. A. Hay, unpublished), at which time tusk size (girth, weight and length) increases rapidly¹⁵. Large numbers of scars on the head occur only on male narwhals longer than 420 cm, well after sexual maturity is attained (Fig. 2).

Of the 39 adult males sampled, 61.5% had broken tusks (distal end broken off), whereas only 10.3% of the 29 juvenile male samples had broken tusks. One of our samples, an adult male, had the tip of a tusk, 9 cm long, embedded in its left upper jaw beside the base of its own tusk. We observed a large scar at the site of embedding and the head was covered with scars. Only one other similar finding has been reported¹⁷. Brown¹⁸ and Porsild¹⁹ occasionally recorded broken tusks with tusk tips jammed into the pulp cavities at the sites of the breaks. Dow and Hollenberg⁶ suggested that the deposition of reparative dentine could account for the tusk 'fillings'. We agree with Porsild¹⁹ that these 'fillings' are tusk tips; the photograph in his paper is convincing. If reparative function accounted for the tusk 'fillings', they should occur more often.

Scars occur in most, if not all, odontocete species²⁰⁻²². Spacing of scars often corresponds with tooth spacing of the same species²¹. Observations of captive odontocetes show that biting and subsequent scarring occur during play, sex and aggression²³. The presence of many scars on the heads of adult males, tusk crossing behaviour, rapid tusk growth at sexual maturity, large proportion of broken tusks in adult males, and the discovery of a tusk tip embedded in the jaw of a male strongly indicate that the tusk is used in intraspecific aggression, most probably during the mating season when male-male competition is expected to be most intense.

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Synaptic mechanisms involved in responses of chromaticity horizontal cells of turtle retina

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Chromaticity-type horizontal cells^{1,2} are interneurons intervening in the first steps of colour-coding information in the vertebrate retina. In turtle retina, the most commonly found of these cells are the red/green horizontal cells (R/G-HCs)³ which are hyperpolarised by green light stimuli and depolarised by red ones. The hyperpolarisation of the R/G-HCs by green light results from a direct input from green cones which are hyperpolarised by green light⁴. The depolarisation of R/G-HCs by red light has been interpreted by Fuortes and Simon⁴ as a consequence of the depolarisation of the green cones due to the activation of a polysynaptic circuit (red cones to L-horizontal cells (L-HCs) to green cones) involving a negative feedback effect of L-HCs on the green cones. Feedback depolarisations of cones result from an increase of the cone calcium conductance which may become regenerative and give rise to spikes^{6,7}. Moreover, prolonged activation of the feedback mechanism of L-HCs on cones in the presence of Sr^{2+} ions in the extracellular medium evokes a repetitive discharge of spikes in cones⁸. In the present work, the effect of Sr^{2+} ions on the responses of both green cones and R/G-HCs have been analysed to test the Fuortes and Simon hypothesis. We have found that in Sr^{2+} media, red light stimuli covering a large retinal area elicit both a repetitive discharge of spikes in the green cones and trains of depolarising transient potentials, probably postsynaptic potentials, in the R/G-HCs. Such results support the idea that feedback depolarisation of the green cones is responsible for the depolarising responses of R/G-HCs to red light.

The experiments were carried out on eyecup retina preparations from the turtle *Pseudemys scripta elegans* placed on a suitable chamber and continuously superfused with a bicar-

bonate saline solution⁹ bubbled with a mixture of 95% O_2 + 5% CO_2 . SrCl_2 was added to the saline without molarity compensation. Intracellular recordings were made with 4 M potassium acetate-filled micropipettes (resistance 200–600 M Ω), connected through an amplifier to a cathode ray oscilloscope and to both tape and pen recorders. The retina was stimulated with a double-beam photostimulator; the light stimuli could be modified by interposition of neutral density and/or interference filters.

As Fuortes *et al.*³ previously described, stimulation with deep red lights covering large retinal fields depolarises the green cones and, in some cases, evokes an initial spike response. This is shown in Fig. 1a₁, where a green cone responded to a large spot of red (700 nm) light by a spike followed by a slight hyperpolarisation. Stimulation of the same area with a 550-nm light evoked a large hyperpolarisation (Fig. 1a₂).

Several minutes after adding 6 mM SrCl_2 to the saline bathing the retina, little change was observed in the responses to green stimuli (Fig. 1b₂), but the large red spot then evoked, almost at the dark potential level, a repetitive discharge of spikes of 25 mV showing large undershoots (Fig. 1b₁). As previously demonstrated^{6–8}, such spikes result from a regenerative increase in conductance across Ca^{2+} channels in the cone membrane and are the consequence of the activation of a feedback connection from the L-HCs⁵. As in the case of all the spikes evoked in cones through the feedback mechanism, the hyperpolarisation of the cone by either inward current injection or appropriate light stimulation can cause partial or total block of the spikes^{7,8}. This is shown for the same green cone in Fig. 1b₃ using a green light stimulus and in Fig. 1b₄ by injecting an inward current pulse.

In the Sr^{2+} -treated retina, in parallel with the appearance of spikes in green cones, trains of transient depolarising potentials were observed in the R/G-HCs. Figure 2 shows records

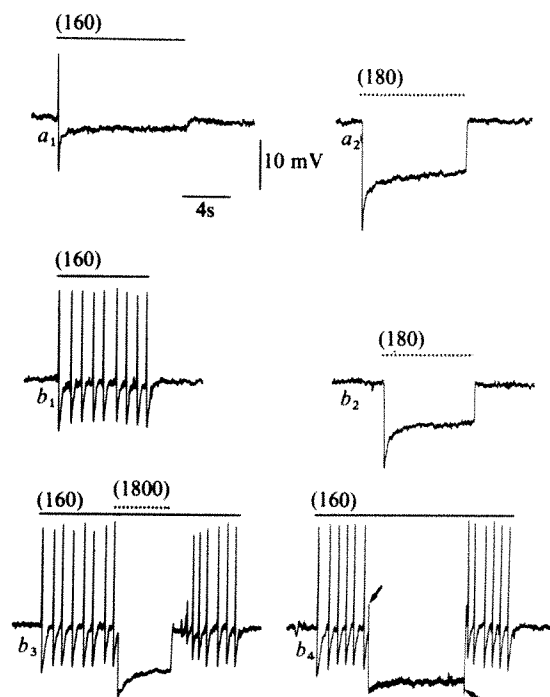


Fig. 1 Responses of a green cone to monochromatic light stimuli. In this and Figs 2 and 3, the stimuli were large spots of 3,700 μm diameter. The solid line traces above the responses indicate the duration of red (700 nm) stimuli, whereas the dotted line traces indicate the green (550 nm) stimuli. The numbers in parentheses, over the stimulus traces when multiplied by 10^3 give the quantum flux in photons $\mu\text{m}^{-2} \text{s}^{-1}$. a₁, a₂, Control recordings in normal saline. b₁–b₄, Responses of the cone during application of 6 mM Sr^{2+} . In b₄ an inward current of 0.2 nA was applied, between the arrows, through the recording microelectrode using a bridge circuit.

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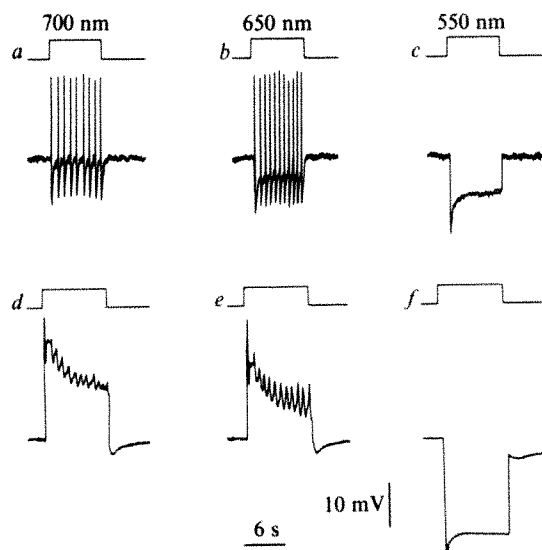


Fig. 2 Responses of a green cone (a-c) and a R/G-HC (d-f) recorded from the same retina bathed in 6 mM Sr^{2+} -containing medium. The wavelength of the light stimuli used in each aligned pair of recordings are indicated at the top. The quantum flux for all stimuli was 170×10^3 photons $\mu\text{m}^{-2} \text{s}^{-1}$.

obtained from a green cone and from a R/G-HC, recorded in the same Sr^{2+} -treated retina. Again, as in Fig. 1, large field stimulation with either 700- or 650-nm wavelength light evoked a repetitive discharge in the green cone (Fig. 2a, b). The same stimuli evoked prolonged depolarising responses in the R/G-HCs (Fig. 2d, e) consisting of an initial peak followed by a plateau phase on which was superimposed a train of transient depolarising potentials showing frequencies similar to those of the spikes in the green cones (Fig. 2d, e). In contrast, stimulation of the same retinal area with 550-nm light evoked only hyperpolarising responses in both the green cones and the R/G-HCs either before or during Sr^{2+} application (Fig. 2c, f).

Similar results were observed in 4 green cones and in 21 R/G-HCs. The frequency of the transient depolarising potentials in the R/G-HCs corresponded to that of the spike discharge in the green cones ($0.5\text{--}2 \text{ s}^{-1}$) when using the same stimuli and their amplitude varied between 2 and 10 mV. In most cases it was possible to show that the depolarisation of the R/G-HC membrane potential beyond the dark level was not necessary for the appearance of the transient depolarising potentials. This is illustrated in Fig. 3a in which large spots of 650-nm light evoked a train of depolarising potentials at a membrane potential close to the dark level. Furthermore, these depolarising transients could also be observed at membrane potentials more hyperpolarised than the dark level. Thus, when the same red stimulus was presented to the cell in the course of an hyperpolarisation elicited by a prolonged green light stimulus it evoked a large prolonged depolarising response whose plateau level did not reach the dark potential. The depolarising transients were, nevertheless, present and their amplitude even seemed to be increased (Fig. 3b). In Fig. 3c the same R/G-HC was stimulated with the same red stimulus during a rather prolonged period during which green stimuli of the same diameter, at different intensities, were intermittently combined with the red light. The two less intense green stimuli elicited hyperpolarising responses and the amplitude of the depolarising transients increased with the cell hyperpolarisation. However, the most intense green stimulus evoked a much larger hyperpolarisation and the disappearance of the depolarising potentials. It also blocked the repetitive spike discharge elicited by 650-nm light in green cones.

In two experiments using two independent microelectrodes, simultaneous recordings could be obtained from both a R/G-HC and a large-field L-HC. In the presence of Sr^{2+} in the

medium no depolarising transients could be evoked in the L-HC by stimuli which induced the appearance of transient depolarising potentials in the R/G-HC. Another point which was carefully investigated in Sr^{2+} -treated retinas was the existence of a possible relationship between the spikes that can be discharged in red cones through the activation of the feedback mechanism⁶⁻⁸ and the depolarising fluctuations evoked by red stimuli in the R/G-HC. We found that depolarising potentials in the R/G-HC could be evoked by stimuli which did not evoke spikes in the red cones, and conversely, stimuli which did evoke spikes in red cones could fail to induce the appearance of depolarising fluctuations in the R/G-HC. These results exclude any correlation between the appearance of feedback spikes in red cones and the depolarising potentials in the R/G-HC.

Our results show that in Sr^{2+} -treated turtle retinas, red light stimuli which elicit repetitive discharge of spikes through the feedback mechanism in green cones also evoke the appearance of depolarising potentials in the R/G-HCs. The spikes in the green cones are due to a regenerative increase in conductance through Ca^{2+} -membrane channels.

What is the nature of the depolarising transient potentials observed in the R/G-HCs? It seems unlikely that these depolarising potentials could be regenerative responses triggered by the sustained depolarisations evoked by red light in the R/G-HCs because such sustained depolarisations are not necessary for the depolarising transients to appear, and because these deflections can be elicited at membrane potentials more hyperpolarised than the dark potential. In contrast, many of the features of the depolarising potentials in the R/G-HCs are consistent with the idea that they are postsynaptic potentials due to phasic transmitter release evoked by the spikes in the green cones. The depolarising transients only appear in Sr^{2+} -treated R/G-HCs and only in response to stimuli which evoke spike discharge in the green cones, the trains of depolarising potentials in R/G-HCs showing a frequency similar to those of the cone spikes and becoming blocked by light stimuli which suppress the spikes in the green cones. Moreover, when the R/G-HCs are hyperpolarised by light stimuli which do not block the spikes in green cones, the amplitude of the depolarising potentials increase. Such behaviour is reminiscent of the classical depolarising synaptic potentials.

The present experiments, therefore, strongly suggest that the depolarising deflections evoked in the Sr^{2+} -treated R/G-HCs

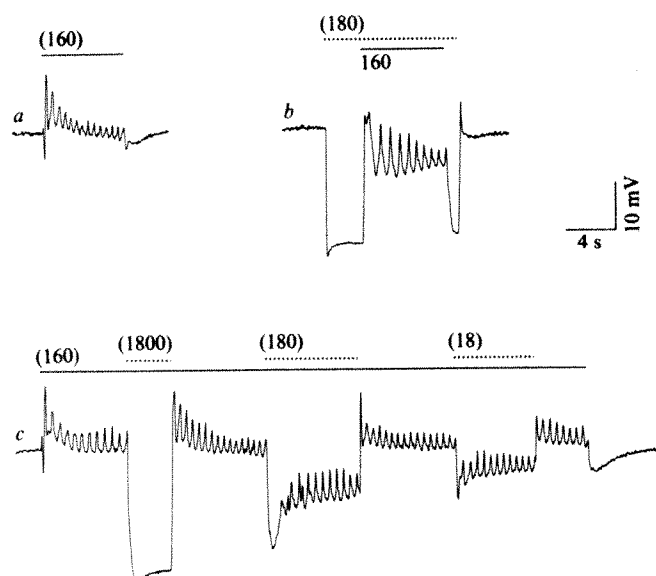


Fig. 3 Responses from an R/G-HC recorded in a retina bathed in a 6 mM Sr^{2+} -containing medium. The full line traces above the recordings indicate the duration of red (650 nm) stimuli whereas the dotted lines indicate the duration of green (550 nm) stimuli. The numbers in parentheses, when multiplied by 10^3 give the quantum flux in photons $\mu\text{m}^{-2} \text{s}^{-1}$.

by red lights are depolarising synaptic potentials secondary to presynaptic spikes evoked through the feedback mechanism of L-HCs on the green cones.

What is the relevance of these results obtained in Sr^{2+} -treated retinas to the known properties of the R/G-HC responses in the normal retina? According to Fuortes and Simon, (1) R/G-HCs receive their only direct input from the green cones; (2) a green stimulus hyperpolarises the green cones, then the R/G-HC; (3) green cones are almost unaffected by red light impinging on them; (4) red illumination of a large area of the retina hyperpolarises the red cones, thus hyperpolarising the L-HCs which receive their main input from red cones; (5) the L-HCs send back their signal on to the green cones, depolarising them, in consequence depolarising the R/G-HCs. The recent anatomical studies of Leeper^{10,11} are consistent with this hypothesis. This author identifies the R/G-HCs with one morphological type of horizontal cell (H2) which is mainly in contact with green cones, and describes another type of horizontal cell (H1-CB) which would be responsible for the feedback effects on the green cones.

From the known properties of photoreceptor synapses¹²⁻¹⁶, hyperpolarisation by green light is expected to suppress the transmitter release from green cones whereas their feedback depolarisation by red light, which involves an increase in Ca^{2+} conductance⁶⁻⁸, is expected to increase the release of transmitter. Our results thus give further support to the hypothesis of Fuortes and Simon, as they indicate that stimuli which evoke depolarising responses in R/G-HCs cause a powerful activation

of the feedback circuit affecting the green cones and when this feedback activation gives rise to a repetitive discharge of Ca^{2+} spikes, depolarising synaptic potentials are actually observed in R/G-HCs. Therefore, the release of a single transmitter from green cones, depressed by the hyperpolarisation evoked by green light and increased by the red light activation of the feedback circuit, can account for the opposite polarity of the responses of the R/G-HCs to such stimuli.

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Postsynaptic effects of ethanol at the frog neuromuscular junction

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The molecular mode of action of alcohol in the central nervous system (CN) is unclear¹. The effects of ethanol on axonal action potentials can only be measured at concentrations which are very much higher than those required to produce central effects². At the frog neuromuscular junction similar concentrations increase the open time (τ) of the ion channel associated with the nicotinic acetylcholine (ACh) receptor³. We have now investigated the effect of ethanol on the postsynaptic membrane of the frog neuromuscular junction by measuring equilibrium dose-response curves for the interaction between the neurotransmitter (ACh) and the ACh receptors. Using this system, we found that ethanol produces significant changes in receptor function. Moreover, we found that at an ethanol concentration which can be physiologically tolerated by man (0.2%) the dose-response curve is measurably affected.

The neuromuscular junction of the cutaneous pectoris muscle in the frog (*Rana esculenta*) is ideal for such studies⁴. The muscle can be dissected to a monolayer of fibres⁵, and the endplate region of the cell membrane which contains the nicotinic ACh receptors can be readily detected with Nomarski optics⁶. The cell membrane is voltage clamped by a conventional two-electrode system and an iontophoresis pipette for the experimental delivery of ACh is placed at a known distance (z) above the finger-like endplate area. A current pulse of known duration and amplitude is applied to the pipette, resulting in the release of a controlled amount of ACh from the tip⁷. The ACh

diffuses to the receptors, thus giving rise to a transient increase in membrane current which is due to the opening of ion channels associated with the receptors^{8,9}. In defined conditions it can be shown that the response is in equilibrium with the concentration of ACh at the receptor site^{10,11}.

Figure 1a shows a typical current response produced by an iontophoretic pulse of ACh in three concentrations of ethanol at the same endplate. The peak current (I_p) in this response was almost doubled in the presence of only 0.2% ethanol. The time to peak (t_p) did not change with the application of ethanol, indicating that saturation of the endplate receptors had not been reached¹⁰. In this condition it was also possible to determine the Hill coefficient (n_H) from the actual time course of the ACh response¹⁰. Such an analysis showed that ethanol had no effect on the value of n_H .

If, in an experiment like that shown in Fig. 1a, one plots the peak amplitude of the response (I_p) against the charge Q (duration multiplied by amplitude of releasing pulse) for different charges, then a dose-response curve is obtained (Fig. 1b). These curves are highly reproducible^{11,12}. From the charge through the pipette it is possible to calculate the amount of ACh which is released and by use of the diffusion equation the concentration profile at the endplate may be defined. From the equilibrium dose-response curve the following parameters may be calculated: (1) the maximum conductance, g_{\max} , per unit length of the terminal (in $\text{nS } \mu\text{m}^{-1}$); (2) the apparent dissociation constant K (in μM) which reflects both the binding and the conformational change of the drug-agonist interaction; (3) the Hill coefficient, n_H . These three parameters completely describe the equilibrium reaction between ACh and the receptors. The evaluation of the parameters of equilibrium dose-response curves is dependent on the use of a kinetic model for the drug-receptor interaction. The model and procedure used here have been explicitly described elsewhere¹², and for an alternative method see ref. 13.

Figure 1b shows a typical ACh dose-response curve obtained before and after the addition of 2% ethanol. The solid lines are the standard curves which provide the best fit to the data. The curve is shifted to the left, signifying that a fixed dose of ACh has a greater effect in the presence of ethanol. It can be seen that the

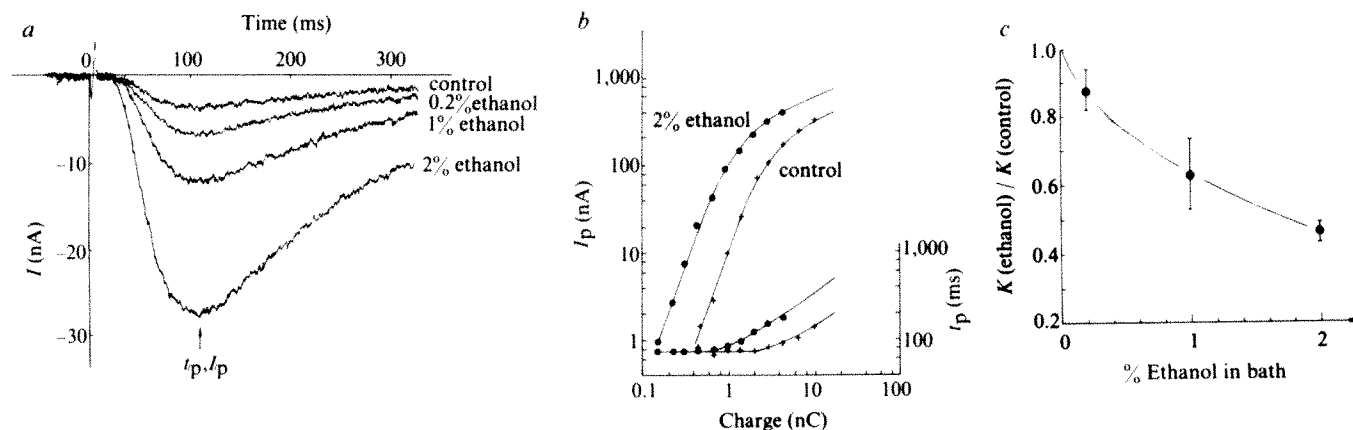


Fig. 1 *a*, Influence of different concentrations of ethanol on endplate currents produced by iontophoretic release of ACh from a pipette located 25 μm above a single linear endplate terminal. The two clamping electrodes were placed on either side of the endplate close to the ACh pipette. The iontophoretic pulse was initiated at time 0. The pulse duration was 1.5 ms and the pulse height 300 nA (charge: 0.45 nC). Ethanol (Merck, 96 vol %) at the above concentrations was diluted in Ringer solution and exchanged into the bath for each measurement. All measurements were obtained at the same endplate in identical conditions. The time to peak (t_p) for each response was unchanged, signifying that the ACh pipette always remained at the same location. The Ringer solution had the following composition (mM): NaCl 115, KCl 2.5, CaCl_2 1.8, phosphate buffer 5, pH 7.1. The membrane potential was -80 mV and the bath temperature was 18°C . The effects of ethanol at any concentration could be reversed by returning to normal Ringer solution whereupon the maximum current (I_p) of the response returned to the control value. *b*, ACh dose-response curves at a single endplate before and after the addition of 2% ethanol to the Ringer solution. The maximum current (I_p) for each response and the associated time to peak (t_p) were plotted against the charge passed through the ACh-releasing pipette (log-log scale). The membrane potential was -80 mV and the temperature was 18°C . The Hill coefficient, n_H (2.8), was measured from the slope of the line at low drug concentrations and was not changed by the addition of 2% ethanol. The solid lines are the theoretically derived curves ($n_H = 2.8$) which permit the calculation of g_{max} and K . The time (t_p , lower trace) to peak current (I_p , higher trace) remains constant for low doses and increases at higher doses. The parameters for the control dose-response curve were n_H 2.8, g_{max} $176 \text{ nS } \mu\text{m}^{-1}$, K $36 \mu\text{M}$, and for 2% ethanol n_H 2.8, g_{max} $168 \text{ nS } \mu\text{m}^{-1}$, K $13.6 \mu\text{M}$ (see Table 1). *c*, Comparison of the apparent dissociation constant K between controls and different concentrations of ethanol for ACh dose-response curves. Each K value was computed for a control dose-response curve and a dose-response curve after the addition of ethanol measured at the same endplate. The ratios were averaged for several different endplates and the graph shows the respective mean values \pm s.e.m. (see Table 1).

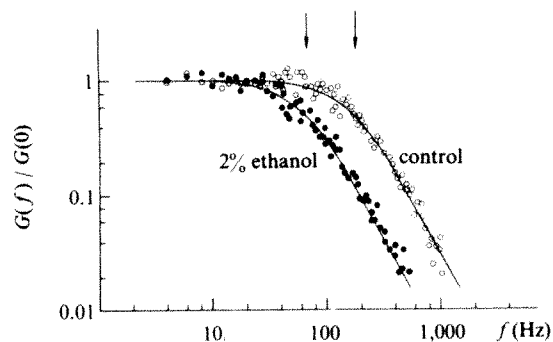


Fig. 2 Normalised power spectrum (log-log scale) calculated from ACh-induced endplate current noise before and after the addition of 2% ethanol at the same endplate. The ACh pipette was located $70 \mu\text{m}$ above the endplate and ACh was continuously released, resulting in a constant mean endplate current, μ_1 . The resulting noise was filtered between 3 and 1,000 Hz, digitised at 2,000 Hz and stored in frames of 1,024 points. The power spectrum was averaged from 20 frames. In each case a background control spectrum in the absence of ACh was subtracted from the ACh-induced spectrum. The solid lines are the theoretical lorentzian functions which provide the best fit to the data. The mean channel lifetime (τ) was obtained from each curve by the formula $\tau = 1/(2\pi f_c)$, where f_c is the cutoff frequency designated by the arrows above each curve. The single channel conductance was obtained from the formula $\gamma = G(0)/[4\mu_1(V_m - V_{\text{eq}})\tau]$, where V_{eq} is the ACh null potential (-5 mV), V_m is the membrane potential (-80 mV) and $G(0)$ is the zero frequency amplitude. The temperature was 18°C . For the control spectra above, $G(0) = 1.67 \times 10^{-22} \text{ A}^2 \text{ Hz}^{-1}$, $\mu_1 = -26$ nA, $f_c = 174$ Hz, $\tau = 0.92$ ms and $\gamma = 23.4$ pS. For the spectra in 2% ethanol, $G(0) = 4.39 \times 10^{-22} \text{ A}^2 \text{ Hz}^{-1}$, $\mu_1 = -29$ nA, $f_c = 66$ Hz, $\tau = 2.4$ ms and $\gamma = 20.9$ pS. Noise analysis at four different endplates before and after the addition of 2% ethanol showed that γ was reduced to 0.91 ± 0.06 (s.e.m.) of the control value and τ was increased to 2.2 ± 0.14 (s.e.m.) of the control value.

slopes of both curves are identical, and thus that the Hill coefficient or cooperativity of the receptor response to ACh is unchanged. This value of 2.7 implies that three or more (perhaps four) ACh molecules are required to bind for one channel to open. The value of K was dramatically reduced, as is evident from the shift in the dose-response curve. In Fig. 1c the relative change in K is plotted against the ethanol concentration, showing that at the lowest concentration of 0.2%, a reduction of 10–20% in K is produced, corresponding to a doubling of the response in the linear part of the dose-response curve.

g_{max} Was not affected by 0.2% or 1% ethanol but was slightly reduced by 2% ethanol (Table 1) which would reflect either a reduction in the total number of channels capable of opening or in the size of the individual channel. This was tested by noise analysis, which showed that indeed the individual channel conductance (γ) was reduced by about 9% and that, in addition, the lifetime of the single channel (τ) was increased in 2% ethanol (Fig. 2). These changes in single channel properties are similar to the findings of Gage *et al.*³. The reduction of γ could, therefore, account for the slight reduction in g_{max} which we have observed in the presence of 2% ethanol.

We therefore conclude that the drastic increase of the post-synaptic response in ethanol as shown in Fig. 1a is due to a reduction in K . Note that even a small change in K will result in a large change in I_p for a constant Q . This effect is due to the steep dependence of I_p on Q , the slope being equal to the Hill coefficient. However, with our method we cannot discriminate between whether only the conformational change or the binding step in the drug-receptor interaction or both have been affected by ethanol.

Dose-response curves are carried out using carbachol as agonist or with ACh after the inhibition of esterase by edrophonium gave a similar reduction in K in the presence of ethanol (Table 1). This effect of ethanol is, therefore, not due to an inhibition of cholinesterase, which could change the apparent dissociation constant. Note that the reduction of K in the

Table 1 Ratio of g_{\max} and K values \pm s.e.m. obtained in different concentrations of ethanol to control values for ACh and carbachol as agonist

Drug	$\frac{g_{\max}(\text{ethanol})}{g_{\max}(\text{control})}$	$\frac{K(\text{ethanol})}{K(\text{control})}$
Acetylcholine		
0.2% ethanol	1.00 ± 0.08 (4)	0.88 ± 0.06 (4)
1.0% ethanol	0.97 ± 0.07 (3)	0.63 ± 0.10 (3)
2.0% ethanol	0.93 ± 0.03 (8)	0.46 ± 0.03 (8)
2.0% ethanol		
+5 μM edrophonium	0.92 ± 0.06 (2)	0.48 ± 0.04 (2)
Carbachol		
2% ethanol	0.92 ± 0.06 (6)	0.62 ± 0.06 (6)

For each data point a dose-response curve was obtained at the same endplate before and after the addition of the corresponding concentration of ethanol. The average values for the parameters of the control dose-response curves were for ACh: $n_H = 2.7$, $g_{\max} = 169 \text{ nS } \mu\text{M}^{-1}$, $K = 27.8 \text{ } \mu\text{M}$ (corrected for ACh-esterase activity) and for carbachol: $n_H = 2.2$, $g_{\max} = 150 \text{ nS } \mu\text{M}^{-1}$, $K = 336 \text{ } \mu\text{M}$ (ref. 10). Numbers in parentheses refer to the number of fibres investigated.

presence of ethanol is greater with ACh as the agonist than with carbachol. As already reported¹², control dose-response curves for carbachol give a lower Hill coefficient, a lower g_{\max} and a higher K than ACh (Table 1). Also, the reduction in g_{\max} in the presence of 2% ethanol is the same as for ACh as for carbachol, showing that the efficacy¹² of carbachol in relation to ACh is not changed.

A reduction of the dissociation constant K by ethanol concentrations as low as 0.2% (a value which can be found in an intoxicated human) could also be demonstrated on mammalian endplates (preliminary experiments on rat and mouse omohyoideus, not illustrated). Here the alcohol effect is even more pronounced.

Ethanol cannot be expected to improve neuromuscular transmission which already has a high safety factor, in healthy specimens. However, it may very well have a positive effect in patients in whom the neuromuscular transmission is impaired (for example, in cases of myasthenia gravis). This positive effect of ethanol could indeed be demonstrated in stimulation electromyography of myasthenic patients, where ethanol concentrations as low as 0.05% proved to be effective¹⁴.

One might speculate that the ability of ethanol to improve the binding of ACh to neurotransmitter receptors could also have highly significant effects in central synapses and thus influence intraneuronal communication which gives rise to normal behaviour.

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Stimulation of hypothalamic nuclei has differential effects on lipid synthesis in brown and white adipose tissue

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The hypothalamus has been implicated in the development of obesity in both humans¹ and experimental animals². The syndrome of hypothalamic obesity results from destruction of the ventromedial region of the hypothalamus³, and investigations of the influences of the hypothalamus on lipid metabolism have been directed chiefly at understanding the mechanism underlying obesity^{2,4,5}. There have been few studies of the effects on lipolysis and lipogenesis in adipose tissue after stimulation of the ventromedial and lateral hypothalamic (LH) nuclei, which may act reciprocally in regulating certain types of peripheral metabolism⁶⁻⁹. We have shown¹⁰ that electrical stimulation of the ventromedial hypothalamic nucleus (VMH) causes lipolysis in adipose tissue, detected by a rapid rise in the plasma glycerol concentration, whereas electrical stimulation of the LH has no such effect. It is not known whether hypothalamic stimulation of either the VMH or the LH affects lipogenesis in adipose tissue. We have investigated this problem by measuring the incorporation of tritium from $^3\text{H}_2\text{O}$ into fatty acids *in vivo*. This is the most reliable method for measuring the rate of fatty acid biosynthesis, because it is independent of the carbon precursor^{11,12}. Surprisingly, electrical stimulation of the VMH enhanced fatty acid synthesis in brown adipose tissue, but not in white adipose tissue or the liver, without the intervention of insulin secretion. Electrical stimulation of the LH, however, had no appreciable effect on lipid synthesis in either type of adipose tissue.

Female Wistar rats weighing about 230 g were housed in individual cages and given free access to laboratory chow and water. The hypothalamus was stimulated, through chronically implanted electrodes, without disturbing the animals' behaviour. Bipolar electrodes of insulated 160- μm wire were implanted stereotactically and unilaterally in the VMH or the LH of rats under pentobarbital anaesthesia. The electrodes were connected to a small plug which was anchored firmly to the skull⁷. The position of the tip of the electrode was verified microscopically in brain sections made when the experiments were complete. Two weeks after implantation, electrical stimuli, consisting of monophasic square pulses of 0.1-0.3 ms duration at 100 Hz with an amplitude of 6 V, were applied to the hypothalamus. Control rats had similarly implanted electrodes but no electrical stimuli were applied. An interval timer was connected to the stimulator to allow repeated stimulation for 30-s periods, once every minute. After 10 min of intermittent hypothalamic stimulation, the rats were injected with $^3\text{H}_2\text{O}$ and 30 min later they were killed. The interscapular adipose tissue (brown fat), the parametrial and retroperitoneal adipose tissues (white fat), and the liver were removed and frozen on dry ice. Total lipids from each tissue were hydrolysed, and after extraction of the fatty acids radioactivity was measured (Fig. 1). In later experiments, some rats were made diabetic (blood glucose levels at the time of death $>400 \text{ mg dl}^{-1}$) by intravenous injection of streptozotocin (65 mg per kg) 24-26 h before hypothalamic stimulation. To minimise the effects of environmental temperature¹³ and of circadian variation¹⁴ on lipogenic activity in adipose tissue, all experiments were performed at $25 \pm 2^\circ\text{C}$ and between 10.00 h and noon, and the animals were not fed for 3 h before the experiments.

The rate of fatty acid synthesis in control rats was about four times greater in brown (interscapular) adipose tissue than in white (parametrial and retroperitoneal) adipose tissue (Fig. 1). With electrical stimulation of the VMH, the rate increased more (about 2.3 times that of unstimulated controls) in the interscapular adipose tissue ($P < 0.05$), but not in the white adipose tissue. Slightly increased rates observed in parametrial and retroperitoneal adipose tissue were not statistically significant ($P > 0.05$). Electrical stimulation of the LH did not appreciably affect lipid synthesis in either type of adipose tissue. Fatty acid synthesis in the liver was not affected by stimulation of the VMH or the LH.

The differential effects of VMH stimulation on lipogenesis in brown and white adipose tissues are probably not mediated by increased secretion of insulin, a pancreatic hormone that stimulates lipid synthesis, because electrical stimulation of the VMH suppressed rather than increased insulin secretion^{8,15}, and if insulin output had increased, fatty acid synthesis would have been enhanced equally in brown and white adipose tissues and liver. When rats were injected with insulin (0.2 U, intraperitoneally), a marked increase in tritium incorporation into fatty acids was observed consistently in all three tissues (data not shown). Table 1 shows the effects of injury to the pancreatic β cells by intravenous injection of streptozotocin before stimulation of the VMH. The appearance of hyperglycaemia (more than 400 mg dl⁻¹) and glucosuria confirmed that insulin secretion was suppressed.

The rate of fatty acid synthesis in the two types of adipose tissue and in the liver of diabetic control rats was reduced to below half the values in healthy control rats, possibly due to the absence of the lipogenic action of insulin. Nonetheless, electrical stimulation of the VMH of the diabetic rats markedly increased lipid synthesis in brown adipose tissue ($P < 0.05$). Again, there was no significant increase in the lipogenic response in either

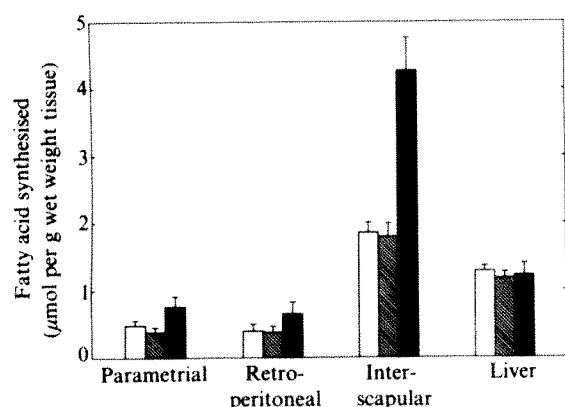


Fig. 1 Effect of hypothalamic stimulation on fatty acid synthesis *in vivo* in adipose tissues and liver. The VMH or LH was stimulated intermittently for 10 min and then the rats were injected intraperitoneally with 3 mCi of ³H₂O in 0.3 ml of 0.9% NaCl solution. After another 30 min period of stimulation, the rats were killed and the interscapular, retroperitoneal and parametrial adipose tissues and the liver were removed and frozen rapidly, and the incorporation of tritium into their fatty acids was measured. Lipids were extracted by homogenising a portion (1 g) of each frozen tissue with 19 ml of chloroform-methanol (2:1, v/v). For isolation of radioactive fatty acids, the lipid extract was washed once with 0.13 M NaCl solution and three times with methanol-0.13 M NaCl-chloroform (50:47:3, by vol), evaporated under nitrogen, saponified with 5 N NaOH at 80°C for 3 h, acidified with 10 N H₂SO₄, and then extracted three times with petroleum ether. The final extract was evaporated and its radioactivity was counted in toluene scintillation solution¹². The rate of fatty acid synthesis was calculated from the radioactivity in fatty acids and in the plasma obtained at the time of death¹⁴. Columns and bars are means and standard errors of values in six to eight animals. Open columns, controls; hatched, LH; solid, VMH.

Table 1 Lipogenic responses of adipose tissues and liver to stimulation of VMH in streptozotocin-induced diabetic rats

	Diabetic control	Diabetic with VMH stimulation
Interscapular adipose tissue	0.48 ± 0.04	1.11 ± 0.12*
Retroperitoneal adipose tissue	0.15 ± 0.01	0.19 ± 0.01
Parametrial adipose tissue	0.16 ± 0.02	0.20 ± 0.02
Liver	0.48 ± 0.05	0.37 ± 0.04

Experimental conditions were as for Fig. 1, except that rats were made diabetic by injection of streptozotocin (65 mg kg⁻¹, intravenously) before they were stimulated electrically. Incorporation of tritium into fatty acids extracted from the adipose tissues and liver was measured, and the rate of fatty acid synthesis was expressed as μmol per g wet weight of tissue in 30 min. Results are means ± s.e.m. of the values in eight animals in each group.

*Significantly different from the unstimulated control group at $P < 0.05$.

retroperitoneal or parametrial white adipose tissue, and conversely fatty acid synthesis in the liver decreased slightly, though not significantly ($P > 0.05$), with stimulation of the VMH.

These results indicate that stimulation of the VMH enhances lipogenesis in brown adipose tissue preferentially, through a mechanism not involving insulin. This effect is probably mediated by sympathetic innervation of brown adipose tissue, because the VMH is presumed to be part of the sympathetic neural output from the hypothalamus^{8,16}. Brown adipose tissue has abundant sympathetic innervation with adrenergic fibres, which form nest-like networks around every fat cell¹⁷. On the other hand, white adipose tissue contains no adrenergic fibres except those related to the blood vessels¹⁸, and consequently it contains much less noradrenaline than does brown adipose tissue. Therefore, noradrenaline may be a mediator of the effect of VMH stimulation on lipogenesis in brown adipose tissue. This idea is supported by preliminary results showing that intraperitoneal injection of noradrenaline (3×10^{-7} mol) into rats increased the rate of fatty acid synthesis in interscapular adipose tissue. However, it is also possible that some lipogenic factor(s) released from the hypothalamus acts on brown fat cells; such a factor has been found in an extract of bovine hypothalamus¹⁹.

Our results, together with the previous observation of increased lipolysis in adipose tissue after stimulation of the VMH¹⁰, suggest that both the breakdown and resynthesis, that is, the turnover, of triglycerides in brown adipose tissue, but not in white adipose tissue, are accelerated by stimulation of the VMH. Because brown adipose tissue is an important fat depot for physiological lipolysis mediated by the sympathetic nerves, especially during cold acclimatisation, this could also explain the increased lipid turnover in the brown adipose tissue of cold-acclimatised animals^{20,21}.

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New class of glutamate agonist structurally related to ibotenic acid

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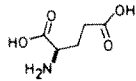
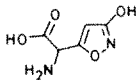
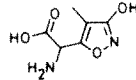
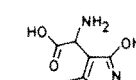
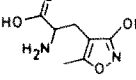
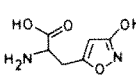
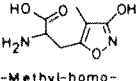
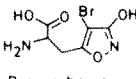
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L-Glutamic acid (Glu) and L-aspartic acid (Asp) are putative excitatory transmitters in the mammalian central nervous system (CNS)¹⁻³. Receptors at Glu- and Asp-mediated synapses are presumably different^{4,5}, and a prerequisite for the identification and characterisation of such sites is the availability of specific antagonists and agonists. Among various potential Glu and Asp antagonists³⁻⁶ Glu diethyl ester (GDEE)⁷⁻⁹ and (D)- α -aminoadipic acid (α -AA)⁹⁻¹³ show some selectivity, the latter particularly towards excitation by *N*-methyl-Asp. Kainic acid (KA), a structural analogue of Glu, is a powerful excitant of CNS neurones¹⁴⁻¹⁶ that seems to interact with only a small proportion of Glu receptors⁵. Ibotenic acid (Ibo) is a powerful neuronal excitant^{9,17,18} also structurally related to Glu. Excitation by Ibo, however, is readily antagonised by α -AA, whereas GDEE has little or no effect¹³, suggesting that Ibo preferentially activates Asp rather than Glu receptors. Furthermore, excitation of neurones by Ibo is followed by a prolonged depression of excitability^{18,19} which is sensitive to bicuculline methochloride¹⁹, indicating that Ibo is probably converted by decarboxylation into muscimol²⁰ during microelectrophoretic ejection near CNS neurones. Thus, neither KA nor Ibo seem to have sufficient specificity to be useful compounds with which to study central Glu or Asp receptors. We describe here a new class of Glu agonist obtained by structural manipulation of Ibo (Table 1). Elongation of the side chain of Ibo by an additional methylene group and introduction of different ring substituents have led to isoxazole amino acids with carboxyl groups resistant to decarboxylation. A further aim of this homologation was to convert the apparent Asp agonist Ibo into a Glu agonist.

Experiments were carried out on lumbar dorsal horn interneurons and Renshaw cells of 11 cats anaesthetised with pentobarbitone sodium (35 mg per kg body weight intraperitoneally, supplemented when required). Extracellular action potentials were recorded by means of the centre barrel of seven-barrel micropipettes, and the compounds tested were administered electrophoretically as anions from aqueous solutions in the outer barrels of the micropipettes²¹. The approximate potency of each excitant was assessed relative to that of Glu on the basis of the electrophoretic current required to produce equal and submaximal excitatory effects on 3 to 19 neurones, taking into account the addition of NaCl (100–165 mM) to dilute solutions (10–50 mM) of strong excitants. Antagonism of amino acid excitation by GDEE and α -AA was tested in each case on 2 to 9 neurones as described previously^{12,13} using Glu and *N*-methyl-(D)-aspartic acid (NMDA) as reference excitants. As in these studies antagonism by either GDEE or α -AA was rarely completely specific, and in Table 1 'yes' indicates a consistent and significantly greater reduction of the effects of a particular excitant by one of the antagonists compared with the other.

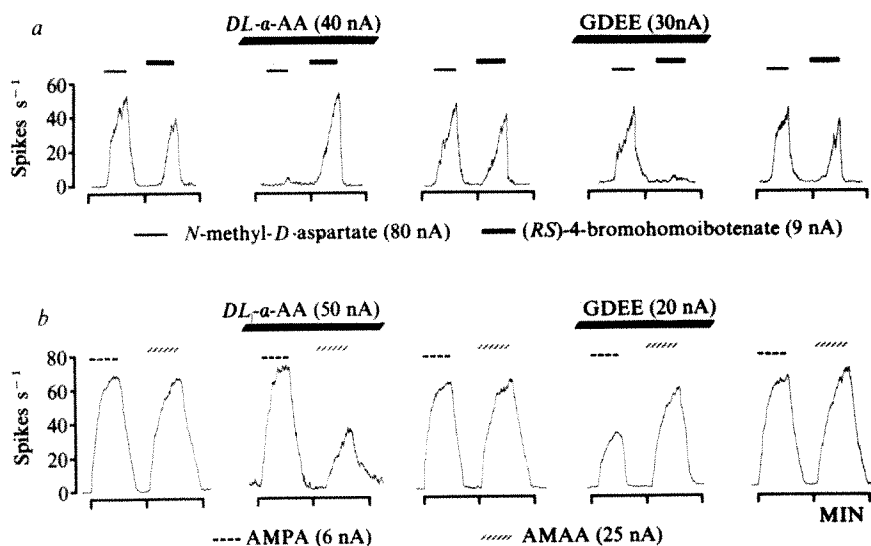
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Table 1

COMPOUND	EXCITATION OF CAT SPINAL NEURONES		INHIBITION OF KAINIC ACID BINDING	
	Rel. potency	Antagonism by GDEE	α -AA	IC ₅₀ values μ M
 (S)-Glutamic acid	++	Yes		0.41
 Ibotenic acid	++++		Yes	12.2
 (RS)-4-Methyl-ibotenic acid	++(+)		Yes	10.0
 (RS)- α -Amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid (AMAA)	++		Yes	>100
 (RS)- α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)	+++++	Yes		>100
 (RS)-Homoibotenic acid	+(+)	Yes		>100
 (RS)-4-Methyl-homo-ibotenic acid	+++	Yes		>100
 (RS)-4-Bromo-homo-ibotenic acid	+++++	Yes		>100

The relative potency of the amino acids as excitants was determined microelectrophoretically as described in the text and shown in Fig. 1. The effects of the excitants are expressed relative to that of Glu (++) by the number of symbols indicating lower, equal, or greater activity, and (+) being of less value than +. Antagonism of the excitatory effects of the amino acids by GDEE or α -AA is indicated by 'yes'. For the ³H-KA binding assay procedure aliquots of rat brain synaptic membranes (0.8–1.2 mg of protein), isolated by a described procedure²³, were incubated in triplicate at 4 °C for 20 min in 2 ml 50 mM-Tris-citrate buffer (pH 7.1), which was 40 nM with respect to ³H-KA, and which contained varying concentrations of KA, Glu, or the listed isoxazole amino acids. After incubation the reaction was terminated by centrifugation for 10 min at 48,000g. The supernatant fluid was discarded, and the pellet was rinsed with two 5-ml portions of ice-cold distilled water. The pellet was suspended in 1 ml of water. To 0.8 ml of this suspension was added 10 ml of scintillation liquid (Insta-gel, Packard), and radioactivity was measured in a liquid scintillation counter (Beckman LS 250). Total specific ³H-KA binding (1,500 c.p.m. per mg of protein) was obtained by subtracting from the total bound radioactivity in the absence of inhibitor (2,225 c.p.m. per mg of protein) the amount not displaced by 100 μ M KA (725 c.p.m. per mg of protein). IC₅₀ values were estimated by examining the effects of at least four different concentrations of the inhibitor and performing log-probit analyses of the results. Log-probit analyses³⁰ and protein measurements³¹ were carried out using published procedures.

Fig. 1 The effects of GDEE and DL- α -AA on the excitation of the firing of two spinal interneurons by NMDA, (RS)-4-bromo-homoibotenic acid, AMPA and AMAA. *a*, NMDA (80 nA, 50 mM in 165 mM NaCl, pH 7), (RS)-4-bromo-homoibotenic acid (9 nA, 200 mM, pH 7), DL- α -AA (40 nA, 200 mM, pH 7), GDEE (30 nA, 200 mM, pH 3.5), all ejected microelectrophoretically as indicated by the appropriate horizontal lines. *b*, AMPA (6 nA, 200 mM, pH 7), AMAA (25 nA, 200 mM, pH 7), DL- α -AA (50 nA, 200 mM, pH 7), GDEE (20 nA, 200 mM, pH 3.5). Ordinates: firing rate, spikes s^{-1} . Abscissae: time, min.



The compounds listed in Table 1 were also tested as inhibitors of the sodium-independent binding of 3H -KA²² to synaptic membranes isolated from rat brains as previously described²³. (RS)-Homoibotenic acid and (RS)-4-bromo-homoibotenic acid²⁴, (RS)- α -amino-3-hydroxy-5-methyl-4-isoxazoleacetic acid (AMAA)²⁵, and (RS)-4-methyl-homoibotenic acid²⁶ were prepared by published procedures. The synthesis of the new compounds (RS)-4-methyl-Ibo and (RS)- α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (see Table 1) will be published elsewhere.

The excitatory action of 4-methyl-Ibo was weaker than that of Ibo and slightly more potent than that of Glu. The effects of Ibo and 4-methyl-Ibo on all three neurones examined were reversibly antagonised by α -AA, whereas GDEE had little or no antagonistic effect. Excitation by 4-methyl-Ibo did not seem to be followed by depression, but as the corresponding decarboxylated compound, 4-methyl-muscimol, is a relatively weak neuronal depressant²⁷, *in vivo* production of this compound may not have been detectable in these experiments. Whereas homoibotenic acid was slightly weaker than Glu as an excitant, its ring-substituted derivatives were much more potent (Table 1). Thus, 4-bromo-homoibotenic acid was more potent than Ibo, which in turn was more potent than 4-methyl-homoibotenic acid. The excitatory effects of homoibotenic acid, 4-bromo-homoibotenic acid, and 4-methyl-homoibotenic acid were completely or partially antagonised by GDEE in a reversible way, but in no case were the excitatory effects of these compounds significantly reduced by α -AA (Fig. 1*a*).

Similarly, AMPA was a GDEE-sensitive, α -AA-insensitive neuronal excitant and was even more potent than 4-bromo-homoibotenic acid (Fig. 1*b*). Whereas AMPA is a Glu analogue, its lower homologue AMAA is structurally related to Asp. Accordingly, the excitatory effect of AMAA, which was similar in potency to that of Glu, was selectively reduced by α -AA. In

general, the neuronal excitations induced by the isoxazole amino acids listed in Table 1 were prolonged compared with that of Glu, suggesting that these compounds were not as effectively removed from the vicinity of the neurones as is Glu. Furthermore, depression was observed only after excitation by Ibo.

In agreement with previous findings, KA^{22,28,29} was found to be a potent inhibitor of 3H -KA binding with an IC_{50} of 0.013 μ M, whereas Glu and in particular Ibo were much weaker (Table 1). With the exception of 4-methyl-Ibo, which like Ibo was a weak inhibitor of 3H -KA binding, none of the isoxazole amino acids studied had any effect in this test system.

These *in vitro* and *in vivo* results suggest that AMPA, homoibotenic acid and its 4-methyl and 4-bromo derivatives excite cat spinal neurones by activating Glu receptors which are different from those on rat brain membranes which bind KA, and that this series of compounds may be useful for future pharmacological analyses of CNS Glu receptors.

In contrast to AMPA, in which the negative charges are separated by five carbon atoms, homoibotenic acid and its derivatives are not strict Glu analogues. Nevertheless, all of these heterocyclic amino acids seem to act on the same receptor, and introductions of ring substituents into homoibotenic acid result in much more potent excitants. The side chains of AMPA and of 4-methyl- and 4-bromo-homoibotenic acid are probably forced by the ring substituents into similar conformations which are readily recognised by the receptors. These steric effects of the methyl groups in AMPA and 4-methyl-homoibotenic acid and the similarity of the proposed resulting conformations are shown in Fig. 2. The depicted orientation of the side chain of AMPA is supported by X-ray crystallographic studies²⁶, and further studies along these lines may elucidate the 'receptor-active conformation(s)' of Glu.

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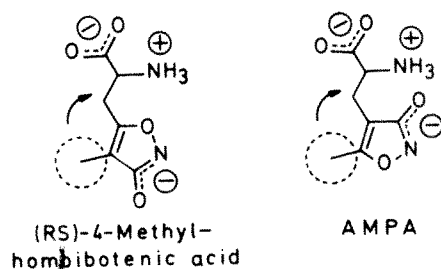


Fig. 2 The structures of 4-methyl-homoibotenic acid and AMPA at physiological pH and their proposed conformations determined by the steric influences of the methyl groups.

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Glucagon and glicentin immunoreactivity are topologically segregated in the α granule of the human pancreatic A cell

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Glicentin is a 100-amino acid polypeptide purified from the porcine intestine¹ and containing the immunodeterminants of glucagon². We have previously reported³ the presence of glicentin immunoreactivity in the glucagon-containing, specific secretory granules (α granules) of the pancreatic and gastric A cell. With an improved immunocytochemical probe, the protein A-gold (pAg) technique⁴, we are now able to show that glucagon and glicentin-like material are topologically segregated in the α granule of the human pancreatic A cell.

Surgical specimens of the pancreatic tail were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4, dehydrated and embedded in Epon 812. Thin sections of the embedded blocks selected for the presence of islets of Langerhans were collected on Parlodion-coated nickel grids and processed for the pAg technique according to the method of Roth *et al.*⁴. The thin sections attached to the grids were incubated for 2 h at room temperature on a drop of anti-glucagon or anti-glicentin serum, washed carefully with phosphate-buffered saline (PBS) and further incubated for 1 h at room temperature with the protein A-gold complex. After a rinse in PBS and distilled water, incubated sections were double stained with 1% aqueous uranyl acetate and 1% lead citrate. The anti-glucagon serum was the 15K antiserum; 15K is a C-terminal anti-glucagon serum which does not react with extracts containing gut GLI's (R. H. Unger, personal communication). 15K was used in a 1:20 dilution. The specific anti-glicentin serum (R64) has already been characterised³, and was used at 1:400 dilution. Protein A-gold solution prepared according to the method of Roth *et al.*⁴ was used at a 1:15 dilution. In some experiments the same thin section was successively incubated with 15K and R64 antisera before exposure to the pAg solution. Thin sections were examined in a Philips EM 301 electron microscope.

The specific secretory granules of the human pancreatic A cell following glutaraldehyde and osmium tetroxide fixation and uranyl and lead staining appear as roundish, membrane-limited vesicles with a non-homogeneous content. Characteristically, the content is formed by a round, electron-dense core surrounded by a mantle of less electron-dense material⁵. This typical morphology is preserved by the glutaraldehyde fixation

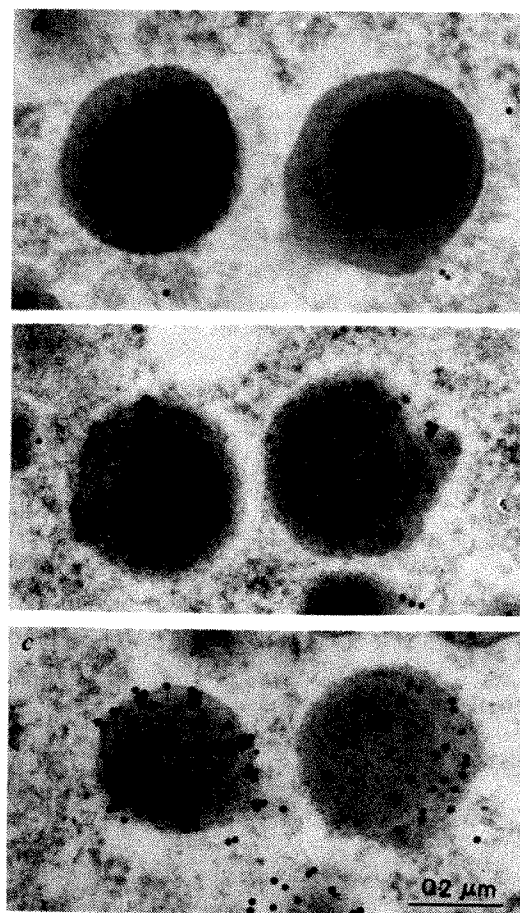


Fig. 1 Three different thin sections showing two human α granules (in near-equatorial section) stained with the pAg technique using 15K anti-glucagon (*a*), R64 anti-glicentin (*b*) and anti-glucagon followed by anti-glicentin (*c*) sera, respectively. All sections were contrasted with uranyl acetate and lead citrate. With the anti-glucagon serum (*a*), the gold particles revealing the site of antiserum binding on glucagon antigens are situated mostly over the roundish, slightly eccentric, electron-dense core of the α granule. The anti-glicentin serum (*b*) binds gold particles almost exclusively over the paler, peripheral mantle of the granule surrounding the dense core. The third granule profile appearing at the lower margin of *b* represents a tangential section through the granule core and shows little gold binding. The three gold particles to the right are interpreted as mantle binding; mantle also appears blurred due to tangential sectioning. The successive incubation with anti-glucagon and anti-glicentin sera (*c*) shows a homogeneous binding of gold particles over the entire surface of the granule. The deposit of gold particles at the lower margin of *c* represents the staining of a tangentially cut (and thus not readily discernible) α granule mantle (compare with *b*). *a*–*c*, $\times 64,000$.

alone used for the pAg technique. In sections incubated with the C-terminal anti-glucagon serum, the gold particles revealing the glucagon antigenic sites seemed to be mostly restricted to the dense core of the α granule (Fig. 1*a*). With the anti-glicentin serum, gold particles were seen mostly on the peripheral mantle (Fig. 1*b*). A quantitative evaluation of the labelling induced by each antiserum on 100 granules showing both the dense core and the mantle in each condition showed approximately 77% of gold particles located over the core in glucagon-immunostained sections and 77% of the particles over the mantle in glicentin-immunostained sections. In sections incubated successively with both specific antisera, approximately equal numbers of gold particles were associated with the dense core and with the mantle of the 100 granules evaluated in this condition (Fig. 1*c*). This labelling pattern was consistently obtained in several experiments with different sections and different blocks of tissue. Control experiments omitting the antiserum step or

carried out with an antiserum absorbed with its corresponding antigen before application of the pAg solution showed only occasional gold particles over some α granules.

Although the presence of glucagon and glicentin immunoreactive material was demonstrated previously in the secretory granules of the A cell by the peroxidase-anti-peroxidase technique of Sternberger⁶ using anti-glucagon and anti-glicentin sera³, a detailed analysis of the localisation of these antigens was not possible due to the masking effect of the dense, irregular deposits of peroxidase reaction product. With the much improved resolution of the pAg technique, glucagon and glicentin immunoreactivity seem not to be mixed in the secretory granule, at least as judged by morphological criteria. In favour of the specificity of mantle labelling following application of anti-glicentin serum is the former cytochemical demonstration that Grimelius' silver preferentially stains the mantle (and not the core) of the α granule⁷, as well as the entire secretory granule content in the L cell⁸, the specific glicentin-producing cell of the intestine³. Although the mechanism(s) by which glucagon and glicentin immunoreactive material are packed and maintained separately in the α granule is unknown, the apparent segregation of these two polypeptides offers a reasonable explanation

of the previously puzzling inhomogeneity of the α granule content. That the dual granule content is co-secreted during α granule release is indicated by recent evidence showing efflux of glicentin-like immunoreactivity by the perfused porcine pancreas (J. J. Holst, S. Lindkaer and A. J. Moody, unpublished data).

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Metastatic potential correlates with enzymatic degradation of basement membrane collagen

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Tumour cells traverse epithelial and endothelial basement membranes during the successive stages of the metastatic process. At the transition from *in situ* to invasive carcinoma, local dissolution of the basement membrane is observed microscopically^{1,2}, and coincides with tumour cell invasion of the underlying stroma. Tumour cells further traverse the endothelial basement membrane during entry into and egress from blood vessels³⁻⁵. Electron microscopy studies have shown local dissolution of basement membrane at its area of contact with invading tumour cells, suggesting an enzymatic mechanism^{3,6,7}. Basement membranes are resilient structures which present a mechanical barrier to invasion⁸. Type IV collagen is a major structural protein of basement membranes and is chemically and genetically distinct from stroma collagen types I and III and cartilage collagen type II^{9,10}. Previously characterised animal collagenases which cleave collagen types I, II and III fail to degrade type IV collagen^{11,12}. We have recently purified about 1,000-fold and characterised a neutral protease activity preferential for type IV collagen from metastatic tumour cells and shown that it (1) produces specific degradation products, (2) has a molecular weight of 65,000, (3) is not plasmin or a cathepsin, by pH and inhibitor studies, and (4) does not significantly degrade other collagens or fibronectin^{12,13}. Here we extend the relevance of this finding by quantitating the ability of several murine tumour cell lines of known metastatic potential to degrade type IV collagen. The cell lines with the highest incidence of spontaneous metastasis exhibit the greatest level of type IV collagen-degrading activity in two different assays using either living cells or media obtained from cell cultures.

The metastatic murine tumour cell lines used here were variants of the B16 melanoma and the T241 sarcoma. Cell lines F₁ and F₁₀ selected from the B16 melanoma exhibit a 10-fold difference in metastatic efficiency^{14,15} (number of lung colonies

per cell following tail vein injection). Line B16-BL6 is a variant with increased invasive capacity *in vitro* and *in vivo* selected from F₁₀ by injecting these tumour cells *in vivo* into the mouse bladder via the vas deferens. The bladder was then excised and cultured in 0.5% soft agar. Colonies in the agar that arose from cells which had invaded the bladder wall were collected and plated in Dulbecco's modified Eagle's medium and 10% fetal calf serum (FCS). When sufficient numbers of cells had grown in culture, they were cycled back through the bladder a total of six times (G. Poste, I. Hart and I. J. Fidler, in preparation). Line

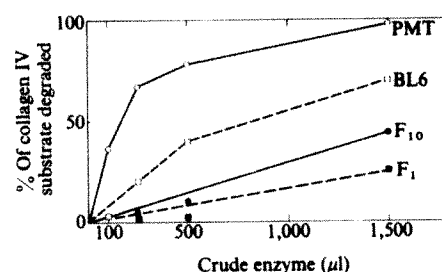


Fig. 1 Basement membrane collagen-degrading activity in culture media from metastatic cell lines. Tumour cells and control cells were grown to log phase in T75 tissue culture flasks. Human polymorphonuclear leukocytes were isolated from the blood of normal volunteers by the Ficoll-Hypaque method, followed by dextran sedimentation and hypotonic lysis to remove erythrocytes. The leukocytes were suspended in Hank's balanced salt solution at a concentration of 4×10^6 cells per ml, and were incubated at 37 °C. Thirty minutes after the addition of FMLP chemotactic peptide, the cells were separated from the medium by centrifugation. The supernatant was assayed for type IV collagen degrading activity. As previously shown¹², 25-50% saturation of ammonium sulphate precipitates virtually all the enzyme activity. The precipitate was dissolved in and dialysed against the reaction buffer (0.05 M Tris-HCl, 0.2 mM NaCl, 10 mM CaCl₂, pH 7.6) in such a volume that it became 20 ml per 10^6 viable cells to assess direct comparison between cell number in original cultures. The reaction was preceded by trypsin activation of the enzyme by 0.25 volumes of trypsin ($10 \mu\text{g ml}^{-1}$) at 37 °C for 4 min followed by trypsin inhibition by 0.25 volume of soybean trypsin inhibitor ($50 \mu\text{g ml}^{-1}$). The enzyme reaction was carried out with various volumes of the activated enzyme at 37 °C for 4 h in a final volume of 1.6 ml containing 50 μl (2,500 c.p.m.) of ¹⁴C-proline-labelled type IV collagen in the reaction buffer. The undigested substrate was precipitated with 200 μl of a solution of 10% trichloroacetic acid and 0.5% tannic acid using 20 μl of bovine serum albumin (1 mg ml⁻¹) as a carrier. After precipitation at 4 °C for 30 min the tubes were spun at 2,500 r.p.m. for 15 min and aliquots of the supernatant were counted in a liquid scintillation counter. Enzyme activity is expressed as radioactivity (c.p.m.) released from substrate per μl of activated enzyme. The relative activity of the cell lines computed from the linear portion of the dilution curve is shown in Table 2. Maximum activity (100% in Fig. 1) is the number of counts released by bacterial collagenase. Trypsin digestions were used to control for any nonspecific protease activity in the samples. Trypsin ($0.5 \mu\text{g ml}^{-1}$) digested 14.0% of the substrate.

Table 1 Degradation of ^{14}C -proline-labelled basement membrane collagen by living cells

Cell type	Type IV collagen degradation (c.p.m. per 10^5 cells)	Incidence of spontaneous lung metastases*	Median (range) no. of pulmonary metastases following tail vein injection (5×10^4 cells) ($n = 10$)
Adult mouse fibroblasts	No detectable activity	None	None
Tumorigenic but non-metastatic transformed fibroblasts	No detectable activity	None	None
Cornea fibroblasts	No detectable activity	None	None
F ₁	35.0 \pm 11.0	None	13 (2-48)
F ₁₀	82.1 \pm 9.0	30% (within 4 weeks)	500 (170-500)
BL6	152.9 \pm 15.5	80% (within 4 weeks)	163 (43-264)
PMT	329.7 \pm 38.0	100% (within 2 weeks)	172 (82-310)

Costar tissue culture cluster wells were coated with ^{14}C -proline-labelled type IV collagen by applying 0.2 ml (2.5×10^3 c.p.m.) of a solution of the collagen in 0.01 M acetic acid to each well followed by evaporation. Cells in log phase of growth were collected by 0.1% EDTA with 80% viability and first washed with complete medium (RPMI-1640 + 10% FCS) and then with serum-free medium. Cell viability was judged by trypan blue exclusion. From 1×10^3 to 3×10^6 viable cells were inoculated into coated wells in a volume of 2 ml serum-free medium. After 10 h 1 ml of medium from each well was collected, centrifuged at 2,000 r.p.m. for 10 min at 4 °C and soluble radioactive digestion products were counted in a scintillation counter. Values shown are based on cell dose-dependent determinations and are related to the number of viable cells. The enzymatic activity is compared with the ability of these cells to form metastases. Both spontaneous lung metastases (derived from the transplanted tumour) and artificially induced lung metastases were studied. Note that the relative activity of the cell lines parallels the spontaneous metastatic properties more closely than the intravenous metastatic assay. Culture medium alone released 45 ± 20 ^{14}C c.p.m. per well or approximately 2.0% of applied counts. Purified bacterial collagenase (10 units) released $2,200 \pm 50$ c.p.m. or 90% of applied counts. Type IV collagen degradation was linear with time over the first 20 h. If soluble counts in an assay did not exceed counts released by culture media alone, the result was scored as no detectable activity.

* After intramuscular injection of 25,000 cells and amputation of the primary tumour ($n = 20$).

PMT was selected from a pulmonary metastasis of the T241 sarcoma as described previously¹⁶. Cell strains ACT and TACT are normal and spontaneously transformed adult mouse connective tissue cells, respectively, obtained by a method first described by Boone¹⁷. TACT cells are tumorigenic in 100% of mice but are non-metastatic.

Our first type of assay for type IV collagen-degradation activity consisted of inoculating living cells into tissue culture cluster wells coated with labelled substrate. The ^{14}C -proline-labelled type IV collagen used as substrate in our assays was prepared biosynthetically in organ cultures of a basement membrane-producing tumour (transplantable EHS sarcoma) from C57BL/6J mice. Methods for purification of the substrate have been described in detail previously^{18,19}. Cells in log growth phase were collected using 0.1% EDTA, and known numbers of viable cells were plated into each well. Degradation of the collagen layer resulted in the release of soluble counts into the medium which, for cells exhibiting activity, was verified in multiple assays to be proportional to the cell number. As shown in Table 1, the tumour cell lines exhibiting the highest activity *in vitro* also exhibited the highest propensity for spontaneous metastasis. However, this assay did not distinguish cell release of collagenase activity from cell phagocytosis of the substrate and intracellular digestion. We therefore searched for type IV collagenolytic activity secreted by these cells. Media from log phase cultures of these cell lines were collected and enzyme activity was precipitated from the media with 25-50% saturation of ammonium sulphate. The enzyme digestion consisted of

native ^{14}C -proline-labelled type IV collagen in solution incubated with trypsin-activated samples at pH 7.6 for 4 h. The undigested substrate was precipitated with 2.0% trichloroacetic acid and 0.08% tannic acid, and the digestion products were counted for radioactivity in the supernatant. This rapid assay for type IV collagenolytic activity, as elsewhere described^{12,13,20}, is highly reproducible. As shown in Fig. 1, the rate of the type IV collagenolytic activity was linear until about 60% of the substrate had been degraded. When the specific activity for each cell line was calculated from the linear portion of the concentration curves, the cell lines with the highest rate of spontaneous metastases *in vivo* exhibited the highest activity (Table 2).

The metastatic process is complex, involving many successive anatomic barriers which must be traversed by the malignant cell and many points where tumour cells can interact with host defences⁴. The ability to degrade basement membranes enzymatically may augment the aggressive behaviour of some metastatic cells. These cells may also differ from non-metastatic cells in other properties such as cell-surface proteins²¹. Furthermore, such an enzymatic activity is probably present in non-malignant cells in conditions where basement membrane is turned over¹². The metastatic tumour cell may exhibit a quantitative difference from normal cells in the synthesis of this enzyme and therefore measurement of its activity might serve as a biochemical marker *in vitro* for the metastatic potential of malignant cells.

We thank Karen Kniska for technical assistance. Bovine smooth muscle, endothelial and cornea cells were supplied by Dr Bert Glaser. We thank Dr G. R. Martin for discussions.

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Table 2 Basement membrane collagen-degrading activity secreted by cultured cells

Cell type	Type IV collagenolytic activity (c.p.m. per 2×10^5 cells)*
1. Mouse fibroblasts	None detected
2. Bovine aorta smooth muscle cells	None detected
3. Bovine cornea fibroblasts	None detected
4. TACT cells†	None detected
5. F ₁	398 \pm 36
6. F ₁₀	714 \pm 62
7. BL6	2,274 \pm 136
8. PMT	8,230 \pm 214
9. Bovine vein endothelium	56 \pm 27
10. Human leukocytes‡	880 \pm 90
11. Human leukocytes + chemotactic peptide‡	2,640 \pm 240
12. MCF-7 Human breast carcinoma cells	1,210 \pm 180

None detected: digestion in the assay was less than trypsin or media controls.

* Lines 1-8, $n = 10$; lines 9-11, $n = 5$. Mean \pm s.d.

† Tumorigenic but non-metastatic spontaneously transformed mouse connective tissue cells.

‡ Experiments in collaboration with Verena Muller and Eliot Schiffman, National Institute of Dental Research, NIH. (V. Muller *et al.*, in preparation.)

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Terminal differentiation in human promyelocytic leukaemic cells in the absence of DNA synthesis

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Some phorbol diesters, the most potent of which is 12-O-tetradecanoylphorbol-13-acetate (TPA), exert two different effects on the differentiation of leukaemic cells *in vitro*: they reversibly inhibit the differentiation in Friend mouse erythroleukaemia cells^{1,2} and mouse myeloid leukaemia M1 cells³, and they induce differentiation in other mouse erythroleukaemia cells⁴, mouse myeloid leukaemia cells⁵, human promyelocytic leukaemia cells⁶⁻⁹ and cells of patients with myeloid and myelomonocytic leukaemia¹⁰. The effect on human leukaemic cells is both irreversible and independent of the continuous presence of the drug⁷. We report here that TPA-induced differentiation of human promyelocytic leukaemia cells (HL60) need not be preceded by a round of DNA synthesis, and that the majority of TPA-treated cells accumulate in the G1 phase of the cell cycle.

We and others have shown previously that TPA-treated HL60 cells 'terminally' differentiate into macrophage-like cells rather than mature myeloid cells^{5,7-9}. The evidence was based on either the appearance of markers in TPA-treated HL60 cells that are specific to the monocytic/macrophage lineage or the disappearance of myeloid markers. One monocytic marker is α -naphthyl acetate esterase (NAE), an enzyme which can be detected in the human bone marrow only in megakaryocytes or in monocytes¹¹; another is the presence of two acid phosphatase isozymes, 3a and 3b (ref. 12), which appear 3 d after TPA treatment⁹. Some myeloid markers, like myeloperoxidase and chloracetate esterase (CAE), are markedly reduced following TPA treatment of HL60 cells⁷. Markers common to both monocytic and myeloid cells, like phagocytosis, immunorethrophagocytosis, Fc receptors for immunoglobulin G, C3 receptors and lysozyme⁵⁻⁸ are also present after TPA treatment of HL60 cells. Although of little use in defining TPA-specific

differentiation, these markers are general indicators of differentiation. The present studies were designed to establish the relationship between the appearance of these differentiation markers and the cell cycle of TPA-treated HL60 cells. Four differentiation markers were used—NAE, CAE, acid phosphatase and its isozymes, and phagocytosis.

Figure 1 indicates that freshly plated, untreated HL60 cells, exponentially growing in culture, do not contain a sizeable fraction of cells in a resting state: continuous labelling with ³H-thymidine (TdR) for 48 h shows that 99% of the cells go through the S phase of the cell cycle at least once. Labelling of the cells for 1 h at the beginning of the experiment shows that about 48% of the exponentially growing cell population is at that time in S.

However, if TPA and ³H-TdR are added simultaneously to freshly plated HL60 cells, only a fraction of the cell population is labelled, even if the cells are exposed to the isotope for 72 h or more. This labelled fraction (52–55%) is only slightly in excess of that obtained by 1-h pulse labelling, which suggests that only a small fraction of the cells (~4–7%) which were not in S at the time of TPA addition are able to enter S after TPA treatment. Instead, most of the cells that were not in S (but in G2, M or G1) will never enter S after TPA treatment. The possibility that the lack of ³H-TdR incorporation in this subpopulation is a result of decreased TdR uptake, associated with normal cell proliferation, is ruled out as only a small increase in cell number is observed shortly after TPA treatment, and there is no increase at all after 12 h (ref. 7). As shown in Table 1, essentially all the TPA-treated cells became intensely positive for NAE (72 h after treatment), and CAE activity disappeared. Acid phosphatase was positive in almost all TPA-treated cells, and analysis of acid phosphatase isozymes by acrylamide gel electrophoresis confirmed that the monocytic-specific bands, 3a and 3b (ref. 9), were induced (not shown). Because about 48% of the TPA-treated cells never entered the S phase, it is clear that acid phosphatase and NAE could be expressed in TPA-treated cells

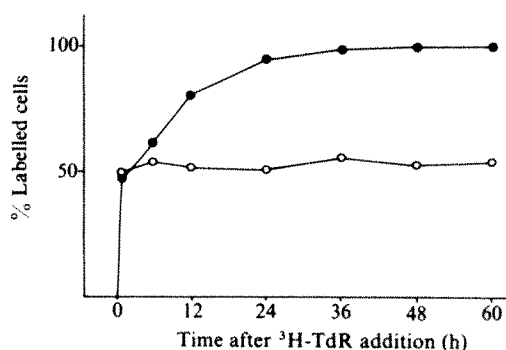


Fig. 1 HL60 cells^{16,17} were grown and induced to differentiate with 1.6×10^{-8} MTPA, as previously described⁷. TPA-treated and untreated cells were continuously labelled with ³H-TdR (0.1 μ Ci ml⁻¹; 6.7 Ci mmol⁻¹), collected by cytocentrifugation at the times indicated in the text and the figure, and processed for autoradiography¹⁵. Percentage of labelled cells are plotted against hours after addition of ³H-TdR. The cumulative percentage of labelled cells was determined at the time indicated. TPA and ³H-TdR were added to the treated cells simultaneously. ●, Untreated cells; ○, TPA-treated cells. Values represent the averages of two different experiments.

Table 1 Percentage of HL60 cells expressing differentiation markers

Experimental conditions	NAE	CAE	AcPh	Phagocytosis	
				³ H-labelled	Unlabelled
Untreated	1	80	9	3	2
TPA-treated	99	4	88	42	40

Cells were collected 72 h after TPA treatment and assayed for α -naphthyl acetate esterase (NAE), naphthol-AS-D chloroacetate esterase (CAE), acid phosphatase (AcPh) and phagocytic activity. A minimum of 500 cells was scored by two independent observers. Values represent averages of duplicate experiments; phagocytosis was scored in conjunction with autoradiography of ³H-TdR-labelled cells to determine whether cells bypassing the S phase developed phagocytic activity. Assay procedures are described in refs 7, 9 and 10.

without the cells undergoing a new round of DNA synthesis. We can also rule out the possibility that these marker enzymes are expressed constitutively in G1 because at any given time, 40–50% of the untreated cells were in G1 (see Fig. 2) but only 1% were positive for NAE and 10% were positive for acid phosphatase (Table 1).

Phagocytic activity was assayed in TPA-treated cells labelled continuously with ³H-TdR for 3 days. Following autoradiography, positive and negative cells were scored for both acid-insoluble radioactivity in the nucleus and the presence of latex beads in the cytoplasm (Table 1). Both the cell populations that went through S and those that did not develop phagocytic capability.

From these experiments, we concluded that transition through the S phase was not required for differentiation of TPA-treated HL60 cells into macrophage-like cells. It was still not clear, however, whether TPA treatment caused cells to stop in different phases of the cell cycle (an indication of a toxic effect on the cells) or in one particular phase. To answer this question, we pulse labelled (1 h) HL60 cells with ³H-TdR at different

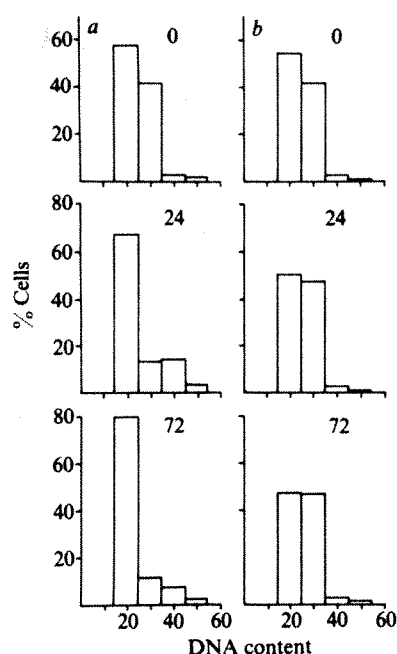


Fig. 2 Percentage of cells plotted against DNA content in arbitrary units. *a* Represents the distribution of DNA content in TPA-treated HL60 cells, and *b*, the distribution of DNA content in untreated HL60 cells. Numbers in the upper right corners indicate hours after plating the cells with or without TPA. HL60 cells have a modal chromosome number of 44 (refs 16, 17). The DNA content of a minimum of 200 cells per time point was determined with a Vickers M85 scanning microdensitometer after Feulgen staining. A standard was obtained by blocking the cells in S phase with fluorodeoxyuridine.

times after the addition of TPA and determined the percentage of cells that incorporated ^3H -TdR into their DNA (Fig. 3). This percentage was found to decrease progressively after TPA treatment. As this result could be interpreted as either a decreasing number of cells in S and normal TdR uptake, or a constant number of cells in S and decreasing TdR uptake, we determined the DNA content in each cell at different times after TPA treatment. The results shown in Fig. 2 indicate that the population of cells in S did, in fact, progressively decrease. The DNA distribution of untreated HL60 cells at different times after seeding was relatively constant: approximately 52% of the cells had a G1-level DNA content, 8% had a G2-level content, and 40% an S-level intermediate content. Following TPA treatment, there is a progressive decrease in the fraction of cells

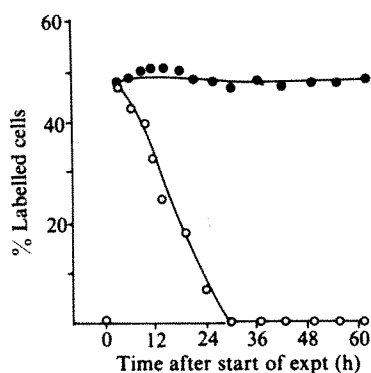


Fig. 3 Percentage of labelled cells plotted against hours after the beginning of the experiment. HL60 cells, either untreated (●) or treated (○) with 1.6×10^{-8} M TPA were pulse labelled for 1 h with ^3H -TdR ($0.1 \mu\text{Ci ml}^{-1}$) at different times after the beginning of the experiment. Values represent the averages of two different experiments.

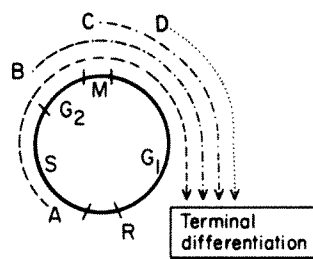


Fig. 4 Cell cycle model for TPA-induced differentiation of HL60 cells. R: point late in G1 beyond which TPA-treated cells may still proceed through one cell cycle. A, B, C, D, Fate of cells in S, G2, M and G1, respectively, at the time of TPA treatment.

with an S-phase DNA content, and an increase in the fraction of cells with a G1 DNA content. Eighty per cent of the cells were found to be G1 72 h after TPA treatment.

These results indicate that TPA-treated HL60 cells accumulate in G1 rather than stopping at various phases of the cell cycle. We have shown previously that the arrested cell cannot be restimulated to proliferate by changing the medium or by removing the TPA⁷. Rather, the cells remain quiescent until their death, 4 to 6 days after the addition of TPA.

The data presented here suggest the model of the TPA effect on the cycle of HL60 cells that is shown in Fig. 4. There is a stage, R, late in G1, after which TPA-treated cells will proceed through S, complete the cycle, stop in G1 and differentiate. Cells in G2, M or early-to-middle G1 at the time of TPA treatment will stop in G1 and differentiate without requiring new DNA synthesis.

The process of TPA-induced differentiation of HL60 cells into macrophage-like cells is rapid and lacks the multiple cell divisions normally associated with the transition from a promyelocytic to a more differentiated myeloid cell. Thus, TPA-induced differentiation differs from known *in vivo* and *in vitro* models of erythroid and myeloid differentiation. However, little is known about monocytic differentiation. It is generally accepted that monocytes and myeloid cells have a common progenitor¹³, and there is evidence for the extremely rapid *in vivo* formation of monocytes from precursors in the mouse¹⁴. Therefore, it is possible that TPA-induced differentiation *in vitro* may not be totally anomalous, but could mimic some *in vivo* process. Because TPA can force myeloid cells in every phase of the cell cycle to become irreversibly quiescent, this drug and others with a similar effect, such as mezerein⁸, may be useful as anti-leukaemic agents in experimental models^{5,6,8}.

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Phorbol ester-mediated stimulation of the synthesis of mouse mammary tumour virus

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12-*O*-tetradecanoyl-phorbol-13-acetate (TPA) is a potent tumour promoter in the two-stage mouse skin carcinogenesis model^{1,2}. Recently, TPA has been shown to enhance the transformation of cultured cells by two DNA tumour viruses—adenovirus³ and Epstein-Barr virus⁴. It is not known whether TPA also augments the transformation of cells by RNA tumour viruses. In Rous sarcoma virus (type C) transformed fibroblasts, the levels of plasminogen activator are elevated by TPA^{5,6}, a phenomenon also associated with malignant transformation^{7,8}. In addition, TPA and other promoters induce the synthesis of some herpesviruses in persistently infected cells^{9,10}. I have investigated the effect of TPA on the synthesis of mouse mammary tumour virus (MMTV), type B RNA tumour virus, and find that TPA, in nanogramme quantities, causes a 10- to 20-fold stimulation of MMTV production by persistently infected mouse mammary tumour cells. This stimulation is specific for TPA; the non-promoter, phorbol, does not stimulate MMTV production.

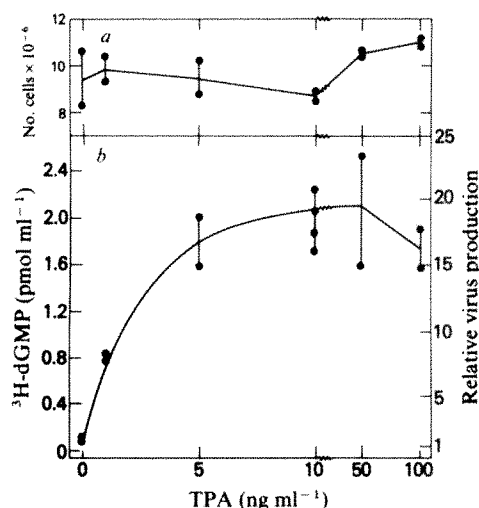


Fig. 1 Effects of TPA on cell growth (a) and virus production (b) by mouse mammary tumour cells grown in the absence of dexamethasone. Duplicate monolayer cultures of Mm5mt/c₁ cells were treated with TPA for 48 h. Twenty-four hours before collecting medium, cultures were given fresh medium with or without TPA. Samples of medium (18–20 ml per culture) were clarified and centrifuged at 25,000 r.p.m. for 90 min at 4°C in a Beckman SW 27.1 rotor. The pellet was suspended in 0.01 M Tris-HCl (pH 7.8)/0.1 M NaCl/1 mM EDTA (TNE), layered on a column of 20% glycerol in TNE, and centrifuged at 35,000 r.p.m. for 60 min at 4°C in a Beckman SW 50.1 rotor. The pellet was dissolved in 100–200 µl of 0.01 M Tris-HCl (pH 7.8)–0.01 M NaCl and 20–40 µl aliquots were used for virus estimation by particle-associated RNA-directed DNA polymerase assay. The polymerase assays utilizing poly(C): oligo(dG) as a template: primer and magnesium ion as a cofactor were performed as described before^{13,14}. The data in (a) show the number of cells per culture and in (b) show the amount of ³H-labelled dGMP (3,500 c.p.m. pmol⁻¹) incorporated into acid insoluble material by purified particle preparation corresponding to 1 ml of collected medium.

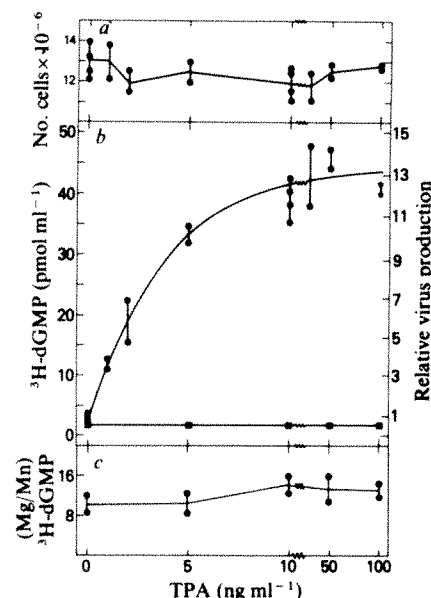


Fig. 2 Effect of TPA (○) and phorbol (■) on cell growth (a) and virus production (b) by mouse mammary tumour cells grown in the presence of dexamethasone (2 µg ml⁻¹). Experimental details as for Fig. 1, except the specific activity of ³H-dGTP used for polymerase assays was 700 c.p.m. pmol⁻¹. The data in (c) show the ratio of magnesium (20 mM) over manganese (1 mM) cofactored DNA polymerase activity of purified particle preparations.

MMTV producing C3H mouse mammary tumour derived cells, designated Mm5mt/c₁ (refs 11, 12), were treated with TPA for 48 h in the presence or absence of dexamethasone. Figure 1 shows the effect of TPA on virus production when the cells were grown in medium without dexamethasone. Clearly, TPA markedly enhanced virus production without significantly affecting cell growth. The effect was dose-dependent: at 1 ng ml⁻¹, the virus production was stimulated by about 8-fold; at 10 ng ml⁻¹, the stimulation was nearly 20-fold. The increased virus production could not be ascribed to the induction of endogenous type C virus(es). The particle-associated DNA polymerase of MMTV prefers magnesium ions as cofactor while that of type C viruses prefers manganese ions^{13,14}. Thus the ratio of polymerase activity in the presence of magnesium and manganese ions is characteristic of the virus type. In our case, TPA did not change this ratio in ways suggesting the induction of type C virus(es) (see Fig. 2c). As dexamethasone also enhances the synthesis of MMTV^{12,15,16}, we determined whether TPA would act synergistically with dexamethasone. As shown in Fig. 2, TPA stimulated virus production even when the cells were grown in the presence of dexamethasone (2 µg ml⁻¹). Again the effect was dose-dependent. The maximum stimulatory effect of 10- to 12-fold was obtained at a TPA concentration of 10 to 20 ng ml⁻¹. As dexamethasone alone stimulated virus production 10- to 20-fold the combined effect of dexamethasone and TPA apparently resulted in 100- to 200-fold enhancement of virus production. This effect was specific for TPA; the non-tumour promoter, phorbol, failed to stimulate virus production up to a concentration of 100 ng ml⁻¹ (Fig. 2).

The effect of TPA on the steady state level of intracellular viral RNA was also examined. This was done by hybridising cellular RNA with representative MMTV complementary DNA (cDNA). The cDNA was prepared by transcribing 30–35S viral RNA with avian myeloblastosis virus reverse transcriptase in the presence of calf thymus DNA oligomers¹⁷. The cDNA contained a uniform representation of 75–80% of viral sequences; other sequences were less frequently represented

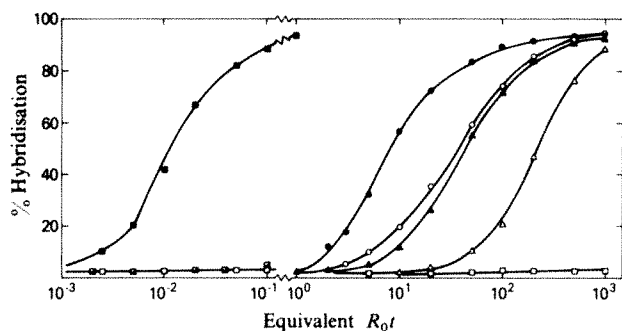


Fig. 3 Effect of TPA on the steady state level of intracellular viral RNA in mouse mammary tumour cells grown in the presence and absence of dexamethasone. Cellular RNA was isolated by phenol extraction and DNase digestion. A solution of RNA ($0.5\text{--}1\text{ mg ml}^{-1}$) and cDNA ($20,000\text{ c.p.m.}$ or 1 ng ml^{-1}) in $0.3\text{ M NaCl}/0.03\text{ M Na citrate } 2\text{ mM EDTA } 0.02\%$ sodium lauryl sarcosinate ($\text{pH } 7$) was incubated at 65°C . Aliquots were withdrawn at intervals and hybrids were scored by S_1 nuclease digestion. The R_0t [concentration of RNA (mol l^{-1}) \times time (s)] values have been reduced to standard conditions of 0.18 M NaCl and 60°C ¹⁸. The curves represent cellular RNA from untreated cultures (Δ), cellular RNA from TPA-treated (20 ng ml^{-1}) cultures (\blacktriangle), cellular RNA from dexamethasone-treated ($2\text{ }\mu\text{g ml}^{-1}$) culture (\circ), cellular RNA from cultures treated with dexamethasone ($2\text{ }\mu\text{g ml}^{-1}$) plus TPA (20 ng ml^{-1}) (\bullet), MMTV RNA (\blacksquare), murine leukaemia virus (AKR) RNA (\blacklozenge), and yeast RNA (\square).

(data not shown). The MMTV cDNA did not hybridise with murine leukaemia virus (AKR) RNA (Fig. 3). Conversely, the cDNA of murine leukaemia virus (AKR) did not hybridise with MMTV RNA. Thus MMTV cDNA contained only MMTV specific sequences. The R_0t analyses revealed that TPA (20 ng ml^{-1}) increased the concentration of intracellular MMTV specific RNA 5- to 7-fold relative to control cultures (Fig. 3). Dexamethasone ($2\text{ }\mu\text{g ml}^{-1}$) alone caused a 6- to 7-fold increase in the concentration of intracellular viral RNA and this was increased a further 5-fold by TPA (20 ng ml^{-1}). These results suggest that TPA causes an increase in the transcription of viral RNA accompanied by a stimulation of assembly and release of virus particles. Note that the magnitude of TPA-caused enhancement of intracellular viral RNA does not strictly correspond to its effect on extracellular virus production. At a TPA concentration of 20 ng ml^{-1} , the level of intracellular viral RNA was increased 5- to 7-fold while extracellular virus production was enhanced 15- to 20-fold. Further, it is not yet known whether TPA directly affects some activity specific for virus synthesis or whether its effect is mediated through some cellular function. The effect of TPA seems to be specific for the synthesis of MMTV; TPA, up to 100 ng ml^{-1} , did not enhance the production of type C virus(es) by late passage Mm5mt/c₁ cells. The biological significance of these results in terms of mammary tumour development in whole animals remains to be ascertained.

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Sister chromatid exchange in cells metabolically coupled to Bloom's syndrome cells

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Cells from persons with Bloom's syndrome (BS) show many more sister chromatid exchanges (SCEs) than cells from normal individuals or those with any other genetic disease studied so far¹. Several other abnormalities demonstrable in cell culture (reviewed in ref. 2) suggest that the molecular defect in BS is related to DNA metabolism; for example, many chromosomal aberrations in untreated cells³, abnormally slow DNA chain growth^{4,5}, and increased sensitivity to treatment with ethyl methanesulphonate^{6,7}. BS cells respond as normal cells do in excision repair⁸⁻¹⁰, 'post-replication repair'¹¹, and repair of single-strand breaks¹². This apparent proficiency at repair prompted the suggestion^{13,14} that the various chromosome abnormalities result from an excessive accumulation spontaneously of lesions in the DNA on which a repair system(s) must act, thereby overloading the system(s). Such lesions might result, for example, from the action of a genetically defective enzyme involved in the semi-conservative replication of DNA, a defect leading to a quantitative imbalance in a chromosomal component, or a defect leading to the intrinsic production of a molecule with mutagenic properties. Experiments designed to test this last possibility have resulted in conflicting reports¹⁵⁻¹⁸. We report here results which do not support the hypothesis that BS cells release such a mutagenic molecule. SCE frequencies in non-BS cells were not affected when the cells were placed in contact-dependent metabolic coupling with BS cells. Also, when we repeated experiments¹⁵ in which medium 'conditioned' by BS fibroblasts was reported to increase the amount of SCE in normal blood lymphocytes, we were unable to duplicate those results.

Lesch-Nyhan syndrome fibroblasts, deficient in hypoxanthine guanine phosphoribosyltransferase (HGPRT⁻ cells), were co-cultivated with BS fibroblasts in hypoxanthine, aminopterin and thymidine (HAT)¹⁹ selective medium. In such conditions, the only HGPRT⁻ cells to proliferate are those metabolically coupled to BS cells²⁰, presumably due to the free exchange of nucleotides through gap junctions²¹. Pairs of cell lines used were opposite in sex—HGPRT⁻ male and BS female. After 3 d of co-cultivation, metaphase chromosome preparations were made. Chromosomes were stained with quinacrine and studied by fluorescence microscopy to determine the presence or absence in each metaphase of a Y chromosome. The chromosomes were then stained to give sister chromatid differentiation, and SCEs were counted. Both experimental and control co-cultivations were performed twice (Table 1, experiments 1 and 2); different lines of HGPRT⁻, BS and normal cells

Table 1 Effect of co-cultivation on SCE frequencies in HGPRT⁻, BS and normal fibroblasts

Cell line(s) in culture		HGPRT ⁻ cells		SCE per cell BS cells		N cells	
Component A	Component B	$\bar{x} \pm \text{s.e.m.}$	Range	$\bar{x} \pm \text{s.e.m.}$	Range	$\bar{x} \pm \text{s.e.m.}$	Range
Experiment 1							
HGPRT ⁻ 1		8.7 \pm 0.5	3-14 (30)				
HGPRT ⁻ 1*		9.3 \pm 0.8	4-17 (24)				
	BS 1			60.0 \pm 2.0	32-80 (30)		
	N 1					10.1 \pm 0.8	3-20 (30)
HGPRT ⁻ 1 + BS 1		10.0 \pm 0.7	5-18 (24)	58.8 \pm 3.1	33-95 (19)		
HGPRT ⁻ 1 + N 1		9.6 \pm 0.5	5-16 (37)			9.5 \pm 0.9	5-16 (18)
Experiment 2							
HGPRT ⁻ 2		8.3 \pm 0.6	4-16 (30)				
HGPRT ⁻ 2*		5.0 \pm 0.4	1-9 (30)				
HGPRT ⁻ 2*		5.8 \pm 0.5	0-14 (30)				
	BS 2			40.8 \pm 1.4	21-56 (30)		
	N 2					8.6 \pm 0.5	4-16 (29)
	N 2*					5.7 \pm 0.4	2-10 (30)
HGPRT ⁻ 2 + BS 2		6.1 \pm 0.4	1-14 (50)	43.7 \pm 1.6	22-58 (29)		
HGPRT ⁻ 2 + N 2		5.8 \pm 0.4	2-12 (26)			6.2 \pm 0.4	1-12 (59)

The fibroblast lines used were actively growing, between the 9th and 22nd *in vitro* passages. Trypsin was used in passaging (1:2 splits) and collection. The HGPRT⁻ cell lines were GM-1662 (referred to as HGPRT⁻ 1) and GM-1362 (HGPRT⁻ 2) obtained from the Human Genetic Mutant Cell Repository at Camden. The BS cell lines were HG 916 (BS 1) and HG 369 (BS 2) derived in this laboratory from skin biopsies taken from patients identified in the Bloom's Syndrome Registry²⁶ as, respectively, 53(StAs) and 26(SaTi). The normal control fibroblast lines (referred to as N in these tables), also derived from skin biopsies taken here, were HG 883 (N 1) and HG 846 (N 2). All co-cultivations were initiated at 1:1 cell ratios. Medium was Eagle's minimal essential medium (MEM) with 10% fetal bovine serum (FBS), penicillin (50 U ml⁻¹), and streptomycin (50 µg ml⁻¹). All medium, except that used for cultures of HGPRT⁻ cells alone, was supplemented with hypoxanthine (2×10^{-2} M), aminopterin (0.8×10^{-4} M) and thymidine (3.2×10^{-3} M) (HAT medium). Cultures were gassed with a mixture of 10% CO₂ in air and stoppered. Initial cell seeding for all cultures was at 3.5×10^5 cells per 8-ounce glass flask. Twenty-four hours after seeding, cells were provided with fresh medium to which 10 µM BrdU had been added. Thereafter, cultures were incubated in the dark until metaphase preparations were made 48 h later by standard techniques. Metaphases from cultures containing two cell lines were stained with quinacrine and studied by fluorescence microscopy. The locations of all diploid metaphases with good morphology were recorded (cells with 43 or fewer chromosomes being rejected) and each metaphase was classified as to the presence or absence of a Y chromosome. Slides were then rinsed in demineralised water and dried overnight. On the following day, the metaphases were stained for sister chromatid differentiation with Giemsa stain, as described previously²⁷. Metaphases previously identified by fluorescence were relocated on the slides and studied for SCEs. SCEs were counted in metaphase cells which had gone through two rounds of DNA replication in the presence of BrdU and which showed good quality sister chromatid differential staining. Metaphases from cultures containing one cell line were stained for sister chromatid differentiation, and SCEs were counted in up to 30 consecutive cells meeting the criteria described above. SCE was determined for some cell lines several times during the course of the experiments, indicated by an asterisk. Numbers of cells analysed are shown in parentheses.

were used in the two sets of experiments. As Table 1 shows, HGPRT⁻ cells in metabolic coupling to BS cells for two cell cycles maintained their normal numbers of SCEs, and they had no effect on SCE in the BS cells.

To determine whether the experimental system had any effect on the amount of SCE present, both lines of HGPRT⁻ cells were co-cultivated in HAT medium with fibroblasts from normal females. The amount of SCE did not differ between co-cultivated and control HGPRT⁻ or normal female cells (Table 1). The HAT medium itself had no effect on SCE in either BS or normal fibroblasts (data not shown).

Published data¹⁵ indicate that medium conditioned by a line of BS fibroblasts increased SCE in phytohaemagglutinin (PHA)-stimulated blood lymphocytes from a normal individual. We repeated that experiment, using 'conditioned' medium from the same BS fibroblast line; also tested were media from two other BS lines and two lines of normal fibroblasts. Medium 'conditioned' by neither BS nor normal fibroblasts affected SCE in the blood lymphocytes of two normal individuals (Table 2).

Data from other laboratories¹⁶⁻¹⁸ indicate that the amount of SCE is reduced in BS fibroblasts in various conditions of co-cultivation, the size of the decrease depending on the initial proportions of cells used. While it is noteworthy that we observed no such effect, the altered metabolism of the non-coupled HGPRT⁻ cells in HAT medium may have precluded it. Thus, while the data presented here do not confirm the reports just cited¹⁶⁻¹⁸, further investigation, designed specifically to study that particular issue, will be required.

Bryant *et al.*²² reported experiments in which cell hybrids between diploid BS and diploid normal fibroblasts were produced; the hybrid cells had the low amount of SCE characteristic of the normal parental cells. With respect to the representation in their genomes of the BS gene, the hybrid cells are the equivalent of tetraploid cells occasionally found in cultures from BS heterozygotes, which have normal numbers of SCEs (our unpublished observations) as do diploid cells from BS heterozygotes^{1,23}. As such, therefore, these hybrids²² give no insight as to whether BS cells produce a factor that can increase SCE in normal cells.

In our experiments (Table 1) every HGPRT⁻ cell showing sister chromatid differential staining was coupled metabolically to a BS cell(s) for two cycles of DNA replication, ensuring exchange of small molecules between the cells²¹. The molecular size limit for exchange through mammalian gap junctions is approximately 900 daltons²⁴, a limit which encompasses essentially all mutagenic molecules currently recognised²⁵. We conclude that no such molecule was transferred from BS to HGPRT⁻ cells. Neither did our results support the idea that BS cells secrete a mutagen of greater than 900 daltons into the culture medium to be taken up by non-BS cells.

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Table 2 The effect on SCE in normal lymphocytes of medium 'conditioned' by BS fibroblasts

Source of medium	SCE in normal lymphocytes			
	Blood no. 1		Blood no. 2	
	$\bar{x} \pm \text{s.e.m.}$	Range	$\bar{x} \pm \text{s.e.m.}$	Range
Control	9.0 \pm 0.6	2-18 (50)	8.4 \pm 0.5	4-19 (50)
N 1	8.2 \pm 1.0	3-12 (12)	7.9 \pm 0.4	2-12 (50)
N 3	9.2 \pm 0.5	4-25 (50)	8.0 \pm 0.6	1-20 (50)
BS 1	9.4 \pm 0.5	2-16 (50)	7.2 \pm 0.5	2-16 (50)
BS 3	8.5 \pm 0.4	2-16 (50)	7.3 \pm 0.4	2-14 (50)
BS 4	10.4 \pm 0.7	2-17 (28)	8.4 \pm 0.5	4-20 (47)

PHA (Wellcome)-stimulated blood samples from two normal individuals were cultured in the dark in 5% CO₂ in Eagle's MEM supplemented with 15% FBS, 2 mM L-glutamine, penicillin (100 U ml⁻¹), streptomycin (100 µg ml⁻¹) and BrdU (25 µM). Forty hours after initiation, cultures handled in a dimly lit room were centrifuged at 1,000 r.p.m. for 8 min and half of the medium (5 ml) was replaced with either fibroblast-conditioned or control (not conditioned) medium prepared as described below. Cells were collected 24 h later for metaphase preparations by standard techniques. Staining for sister chromatid differentiation and criteria for cell selection were as for Table 1. Slides were coded, and SCEs were counted directly through the microscope ($\times 1,000$) in up to 50 consecutive metaphases. Fibroblast medium was as described for Table 4, experiment 2 of ref. 15 except that antibiotic concentrations were 100 U ml⁻¹ for penicillin and 100 µg ml⁻¹ for streptomycin. All fibroblast cultures were actively growing, between subculture passages 16 and 29 (1:2 splits). The final passage was by a 1:4 split, and 24 h later fibroblasts were rinsed with Earle's balanced salt solution and then provided with fresh medium. After further 4 d of incubation, medium was removed from nearly confluent cultures and centrifuged at 1,200 r.p.m. for 10 min to remove cellular debris. BrdU (25 µM) was added to both the conditioned media and the control medium, and these media were used immediately to replace half of the medium in the blood cultures. BS fibroblast lines used were HG 916 (BS 1), HG 1219 (BS 3) and GM-1492 (BS 4) from, respectively, 53(StAs), 81(MaGrou) and 44(AbRu). Control fibroblast lines were HG 883 (N 1) and HG 978 (N 3). Cell lines BS 1 and N 1 are the same as in Table 1. All cell lines were derived from skin biopsies taken in this laboratory, except GM-1492 which was obtained from the Human Genetic Mutant Cell Repository. Numbers of cells analysed are shown in parentheses.

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Induction of sister chromatid exchanges by BUdR is largely independent of the BUdR content of DNA

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The halogenated thymidine (dT) analogue, 5-bromodeoxyuridine (BUdR), has a variety of effects on mammalian cells, including toxicity, suppression of differentiation, and mutagenesis. Although it is generally assumed that the effects of BUdR are due primarily to its presence in DNA, results from our laboratory have raised doubts about such assumptions¹⁻⁴. We have shown, for example, that BUdR mutagenesis in mammalian cells is determined by the concentration of BUdR in the medium rather than in DNA³, and that mutagenesis can be suppressed by deoxycytidine (dC) without changing the amount of BUdR in DNA⁴. BUdR has also been shown to induce sister chromatid exchanges (SCEs) in mammalian cells⁵⁻⁷. Initial results suggested that the relationship between BUdR and SCEs might not be explained by a single factor⁵, and various correlations between BUdR and SCEs have been proposed^{8,9}. However, the results to date have been inconclusive, because the experiments did not resolve as independent variables the concentration of BUdR in the medium and the amount of BUdR incorporated into nuclear DNA. We have now carried out experiments to resolve these two factors; the results indicate that the major factor in determining the frequency of SCEs is the concentration of BUdR in the medium.

The cells used in these experiments were from a clone of Chinese hamster ovary (CHO) cells. The occurrence of SCEs was monitored by growing cells for two generations in the presence of BUdR and staining with the fluorescent bisbenzimidazole dye, 33258 Hoechst¹⁰, and Giemsa¹¹. In most experiments, the incorporation of BUdR into nuclear DNA and the induction of mutations by BUdR were measured in parallel cultures.

When CHO cells were grown in medium containing BUdR at concentrations ranging from 12 to 300 µM, the frequency of SCEs increased by over fourfold and the level of BUdR incorporation into DNA (BUdR substitution) also increased (Fig. 1a). To evaluate the role of the increased BUdR concentration *per se*, cells were grown in medium (FBT) containing 5-fluorodeoxyuridine (FudR, an inhibitor of *de novo* dT biosynthesis) plus BUdR and dT at varying concentrations but at a fixed ratio. When CHO cells were grown in FBT medium containing 12-300 µM BUdR and with the BUdR:dT ratio fixed at 3:2, the frequency of SCEs increased by over threefold (Fig. 1a). However, because of the presence of FudR and the fixed ratio of BUdR:dT, the level of BUdR substitution remained constant at approximately 60%. The increase in SCEs due to raising the BUdR concentration at a fixed level of BUdR substitution accounted for most of the increment in SCEs observed when the level of BUdR substitution was allowed to increase with the BUdR concentration. Similar data were obtained in several independent experiments. These results suggest that the major factor in determining the frequency of induction of SCEs is the concentration of BUdR in the medium rather than the amount of BUdR incorporated into DNA. The

Table 1 Induction of SCEs by BUdR

Medium additive (μ M)		BUdR substitution (%)	SCEs per cell	Mutant frequency
BUdRdT	dC			
Part 1: FUDR present				
12	24	38.8	7.5	<1
180	360	35.0	23.7	120
12	8	64.5	10.4	<1
180	120	59.1	22.6	214
12	2	85.0	12.9	<1
180	30	85.8	24.2	168
Part 2: FUDR present				
12	24	33.9	6.6	<1
12	8	64.5	9.3	<1
12	2	84.1	11.4	<1
12	—	95.1	14.4	NT
Part 3: FUDR absent				
12	—	75.8	8.7	<1
180	—	94.0	23.4	152
Part 4: FUDR present				
12	24	—	8.2	NT
12	24	200	7.9	NT
12	1	—	12.7	NT
12	1	200	13.3	NT

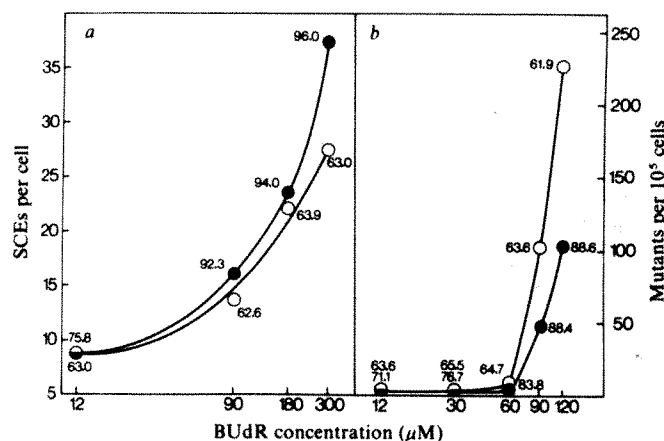
The analyses were carried out as described in Fig. 1 legend. The cells were exposed to BUdR in either FBT medium (FUDR present) or E medium (FUDR absent) with the indicated concentrations of BUdR, dT and dC. NT, Not tested.

induction of mutations by BUdR in CHO cells was also determined by the BUdR concentration in the medium (Fig. 1b), in agreement with the results of our previous studies with Syrian hamster cells³. The parallel between the induction of SCEs and mutations by BUdR agrees with the relationship previously proposed for SCEs and mutations induced by other agents¹².

The results in Fig. 1a showed that increasing the BUdR concentration while holding the level of BUdR substitution constant at 60% resulted in a large increase in the frequency of SCEs. The ratio of BUdR:dT in FBT medium was varied, and similar tests were carried out with the level of BUdR substitution fixed as low as 35% or as high as 85%. As shown in Table 1, part 1, the relationship between BUdR concentration and SCEs was observed over the entire range of BUdR substitution levels tested.

Experiments were carried out to determine whether increasing the level of BUdR substitution had an effect of its own on the induction of SCEs. CHO cells were grown in FBT medium with a fixed concentration of BUdR (12 μM), and the level of BUdR substitution was increased by decreasing the amount of dT in the medium. As shown in Table 1, part 2, increasing the BUdR substitution in this manner from 34% to 95% had only a small (approximately twofold) effect on the frequency of SCEs, although this effect was reproducible in independent experiments. These results suggest that the level of BUdR substitution has a minor role in determining the frequency of SCEs induced by BUdR. The relative roles of BUdR concentration and substitution in determining the frequency of SCEs can be judged by comparing the SCEs induced by 180 μM BUdR alone with those induced by FUDR plus 12 μM BUdR but without dT. Although the levels of BUdR substitution were the same in both conditions (~95%), the frequencies of SCEs were quite different (Table 1, parts 2 and 3). Clearly, the concentration of BUdR, not the level of BUdR substitution, was the major factor. Furthermore, as shown in Fig. 2, the ratio of exogenous BUdR molecules:cell number did not have a significant effect on the frequency of SCEs, in contrast to previous reports^{8,9}.

The results in Table 1, part 2, showed that increasing the level of BUdR substitution while maintaining the concentration of

**Fig. 1** Effect of BUdR concentration on the induction of SCEs.

For the determination of SCEs, tissue culture dishes (100 mm diameter) were inoculated with 5×10^5 CHO cells in 9 ml of Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum (E medium). After several hours at 36.5 °C, 1 ml E medium containing BUdR and other additives at 10 times the desired final concentration was added to each dish. After 36 h, colcemid ($0.06 \mu\text{g ml}^{-1}$) was added, the cells were collected 2 h later, stained with 33258 Hoechst dye¹⁰, exposed to visible light and stained with Giemsa as described previously¹¹. Each point represents the average frequency of SCEs per cell for at least 20 cells. For the determination of mutant frequencies, dishes (100 mm diameter) were inoculated with 1×10^5 cells in 9 ml E medium, and the cells were exposed to BUdR and other additives for 36 h as described above. After the exposure to BUdR, the cultures were rinsed twice and renewed with 20 ml E medium containing 0.1 mM hypoxanthine and 16 μM dT (HT medium). After 5 days growth in HT medium, to allow for the expression of any mutations induced, the cells were collected and dishes (150 mm diameter) were inoculated with 2×10^5 cells in E medium containing 18 μM 6-thioguanine (TG). Dishes (60 mm diameter) were also inoculated with 250 cells in E medium alone. After 7 days, the cultures were fixed and stained, and colonies with more than 50 cells were counted in triplicate dishes. The results are expressed as the number of TG^r colonies per 10^5 cells, after correcting for the plating efficiency of the cells in the absence of TG. For the determination of BUdR incorporation into DNA, dishes (150 mm diameter) were inoculated with 10^6 cells in 45 ml E medium. After several hours, 5 ml E medium containing BUdR and other additives at 10 times the desired final concentration was added. Twelve hours later, $2 \mu\text{Ci ml}^{-1}$ $\text{H}_3^{32}\text{PO}_4$ was added. After a further 36 h, the cells were collected, the DNA isolated and the base composition of the nuclear DNA determined as previously described¹⁷. Four determinations were made for each sample. The results are expressed as 'BUdR substitution', the percentage of dT residues in nuclear DNA replaced by BUdR. To avoid effects due to the photosensitivity of BUdR substituted DNA, the cells were protected at all times from wavelengths of light below 550 nm¹⁷. The cells were exposed either to BUdR alone (●) or to BUdR in the presence of 10 μM FUDR and dT (FBT medium) with the ratio of BUdR:dT fixed at 3:2 (○). The numbers by the data points indicate % BUdR substitution.

BUdR fixed had a small but apparently real effect on SCE induction. However, it can be seen that increasing the level of BUdR substitution in these conditions had no detectable effect on the induction of mutations. Thus, even though a reasonably good correlation between the induction of SCEs and mutations by BUdR was observed in most conditions (see Figs 1, 3), conditions exist in which the induction of SCEs and mutations by BUdR seem to be separable.

Because we had found that dC can suppress BUdR mutagenesis⁸, experiments were carried out to determine the effect of dC on BUdR-induced SCEs. These experiments were carried out in the presence of FUDR, to prevent the conversion of the exogenously supplied dC to dT nucleotides. As shown in

Fig. 3a, the addition of 200 μM dC largely suppressed the increase in SCEs due to increasing the BUdR concentration from 12 to 300 μM . Because of the presence of FUDR, this suppression occurred in the absence of changes in BUdR substitution. Comparable results were observed for the suppression of BUdR mutagenesis by dC (Fig. 3b), in agreement with previous results⁴.

Experiments were also carried out to test the ability of dC to suppress the induction of SCEs caused by increasing the level of BUdR substitution (from 35% to 88%) while maintaining a fixed concentration of BUdR (12 μM). In contrast to the results in Fig. 3a, dC had no effect on the induction of SCEs by BUdR in these conditions (Table 1, part 4). Thus, dC can suppress the induction of SCEs caused by increasing the concentration of BUdR, but apparently cannot suppress the induction of SCEs caused by increasing the level of BUdR substitution.

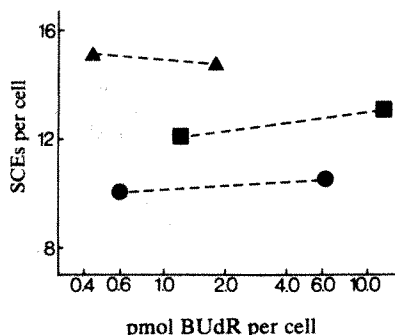


Fig. 2 Effect of BUdR:cell ratio on the induction of SCEs. The analyses were carried out as described in Fig. 1 legend. The cells were exposed to BUdR in FBT medium with a 3:2 ratio of BUdR:dT. At a given BUdR concentration, the different BUdR:cell ratios were generated by varying the ratio of the volume of culture medium to the number of cells. At 30 μM BUdR (●) and 60 μM BUdR (■), cultures were tested at 5×10^5 cells per 10 ml (left point) or 1×10^5 cells per 20 ml (right point). At 90 μM BUdR (▲), cultures were tested at 1×10^6 cells per 5 ml (left point) or 5×10^5 cells per 10 ml (right point).

As the BUdR plus dye technique is being used to screen compounds for SCE induction, experiments were carried out to determine whether the conditions of BUdR treatment could affect the induction of SCEs by other agents. Cells were grown in FBT medium such that the frequency of SCEs was varied by changing either the BUdR concentration at a fixed BUdR substitution (60%) or the BUdR substitution at a fixed BUdR concentration (12 μM). The effect of mitomycin C (15 or 30 ng ml^{-1}) in inducing SCEs in the various conditions was determined, and in no case was there evidence for interactions between BUdR and mitomycin C. For example, in FBT medium with a 3:2 ratio of BUdR:dT, 30 ng ml^{-1} mitomycin C caused an increase of 96.9 and 95.3 SCEs per cell above the baseline for BUdR at 12 μM (9.3 per cell) and 180 μM (23.3 per cell), respectively. These results agree with those of a previous study in which BUdR concentration and substitution effects were not tested separately¹³.

The results presented above show that (1) the major factor in determining the frequency of SCEs induced by BUdR is the concentration of BUdR in the medium, (2) the amount of BUdR in DNA has only a small role in determining the frequency of SCEs, (3) the induction of SCEs by high concentrations of BUdR can be suppressed by dC without changing the amount of BUdR in DNA, and (4) the induction of SCEs by high levels of BUdR substitution is not suppressed by dC. Overall, there was a reasonably good correlation between SCEs and mutations,

although conditions to separate these two effects of BUdR were found.

The specific mechanism by which BUdR induces SCEs remains to be elucidated. However, the results presented above suggest that the primary effect of BUdR in inducing SCEs may

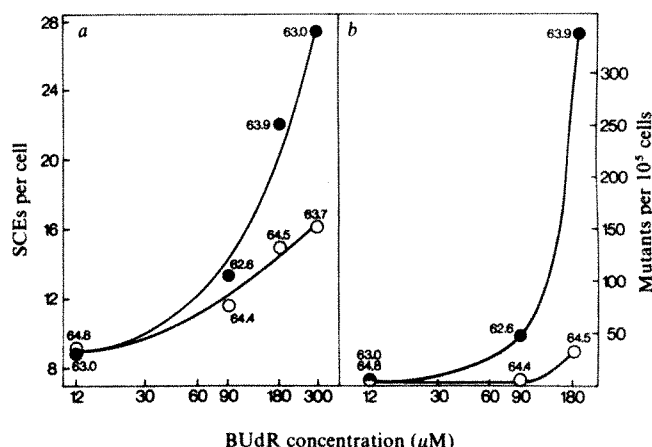


Fig. 3 Effect of deoxycytidine on the induction of SCEs by BUdR. The analyses were carried out as described in Fig. 1 legend. The cells were exposed to BUdR in FBT medium with a 3:2 ratio of BUdR:dT. Half the cultures received 200 μM dC (○) at the same time that BUdR was added, the other half received no dC (●).

occur at a site outside DNA (although the ultimate target for SCEs is obviously DNA). One possibility raised by our results is that perturbation of dC metabolism has a key role in the induction of SCEs by high concentrations of BUdR, as previously suggested for BUdR mutagenesis^{2,3,14,15}. The induction of SCEs and mutations by high BUdR concentrations could involve a common site outside DNA, possibly the inhibition by BUdR triphosphate¹⁶ of the ribonucleotide reductase-catalysed reduction of CDP to dCDP. The fact that dC did not suppress the (small) increase in SCEs caused by increasing the level of BUdR substitution suggests that the independent effects of BUdR substitution and concentration on SCEs might involve different mechanisms. Of the two components of the effect of BUdR on SCEs, the minor component (BUdR substitution) may be directly related to the presence of BUdR in DNA, whereas the major component (BUdR concentration) seems to involve other sites.

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Influence of local vascularity on hormone receptors in mammary gland

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Procedures, such as teat removal (thelectomy) or teat duct ligation, which prevent removal of milk, lead to rapid involution of the lactating mammary gland; performed unilaterally they have been used previously to study the biochemistry of involution¹⁻³, enabling a comparison of normal and involuting glands in the same animal against the same systematic hormonal environment. Both the protein hormone prolactin and the steroid hormone oestrogen are of importance in the development and function of the mammary gland⁴. In the present experiments, female Sprague-Dawley rats were unilaterally thelectomised and the binding to the mammary gland of prolactin and oestrogen was examined through pregnancy, lactation and weaning. There was an effect of thelectomy during lactation only, when levels of both receptors increased in the intact lactating gland but failed to rise in the thelectomised, involuting gland. Capillary closure is known to occur in the mammary glands of rats after 36–48 h of milk accumulation⁵. The rate of delivery of hormones to the tissue will be drastically reduced and it is concluded that this, rather than systemic hormone levels, is of importance in controlling receptor levels.

Female Sprague-Dawley rats aged 34–45 days were unilaterally thelectomised under ether anaesthesia by ligation of milk ducts and excision of teats. Rats were allowed to recover for at least 21 days, and were then placed with a male for 4 days. The stage of pregnancy was confirmed at autopsy by inspection of pup development. The day of littering was day 1 of lactation. Litters were adjusted to 6 pups per dam and pups were weaned on day 21 of lactation. All animals were killed by stunning and immediate decapitation between 09.00 h and 11.00 h (lights on 08.00–20.00 h). Because of the small amounts of mammary tissue in virgin and some early pregnant females, mammary tissue from two or three animals was pooled for hormone binding measurements at these stages. Mammary glands were removed, placed on ice, blotted and weighed. The wet weight of mammary tissue reflected expected developmental changes, rising slightly between early and late pregnancy and increasing a further twofold in lactation in intact glands. Mammary weight differed between intact and thelectomised glands only during lactation. Thelectomised glands were about 60% of the weight of intact glands between 2 and 7 days post partum, falling to 40% in late lactation, equal to the weight in weaned rats, thereby demonstrating progressive involution.

To measure hormone receptors, mammary tissue was immediately cut into small pieces and homogenised in 3 volumes (w/v) of ice-cold buffer (0.01 M Tris-HCl, 0.0015 M EDTA, 0.012 M thioglycerol, pH 7.4, containing 10% glycerol, v/v), using 10-s bursts of a Silverson laboratory mixer-emulsifier at maximum speed with 30-s cooling periods until no solid material remained (three to six bursts). The total homogenate was centrifuged at 15,000g for 20 min (4 °C), the pellet and floating fat were discarded and the aqueous supernatant was centrifuged at 100,000g for 1 h. The supernatant (cytosol fraction) and the pellet (membrane fraction) were stored at –20 °C until assayed for oestradiol binding and prolactin binding respectively.

Table 1 Specific binding of ¹²⁵I-labelled ovine prolactin to intact and operated mammary glands of unilaterally thelectomised rats

Rats	¹²⁵ I-ovine prolactin bound (c.p.m. per 0.1 g wet weight mammary gland)		Significance
	Intact mammary gland	Thelectomised mammary gland	
Virgin (n = 4)	872 ± 193	643 ± 296	NS
Early pregnancy (1–11 days) (n = 6)	863 ± 538	1,585 ± 1,208	NS
Late pregnancy (12–22 days) (n = 9)	2,615 ± 2,054	1,768 ± 1,429	NS
Early lactation (2–7 days) (n = 6)	5,394 ± 1,446*	1,312 ± 1,109	P < 0.002
Late lactation (8–21 days) (n = 7)	6,531 ± 1,956	1,050 ± 778	P < 0.001
Weaned (5–12 days) (n = 6)	906 ± 808†	588 ± 338	NS

Prolactin binding was assayed in the membrane fraction (100,000g pellet) as previously described⁶. Ovine prolactin (NIH-P-S6) was iodinated to a specific activity of 22–48 µCi µg⁻¹ and 40,000 c.p.m. added to each assay tube. Values are means ± s.d.; n = no. of observations; NS, not significant, P > 0.05, Student's *t*-test.

* P < 0.001 versus intact mammary gland of virgin rats.

† P < 0.001 versus intact mammary gland of lactating rats.

The binding of prolactin (Table 1) and of oestradiol (Table 2) were measured in intact control mammary glands and in the contralateral thelectomised mammary glands of 9 virgin, 17 pregnant, 13 lactating and 6 weaned rats. Glands from virgin females showed low levels of prolactin and of oestradiol binding and there were no significant effects of thelectomy. Binding remained low during the early stages of pregnancy. After mid-pregnancy, prolactin binding tended to increase more in intact than in thelectomised glands but there were no significant differences between intact mammary glands of rats in early and late pregnancy or between intact and thelectomised mammary glands in late pregnancy. Oestradiol binding tended to be lower in late pregnant than in virgin females in both intact and thelectomised glands.

From the onset of lactation there were highly significant differences in prolactin binding between intact and thelectomised mammary glands. By 2–7 days post-partum, prolactin binding had increased in intact glands to two to three times the levels in late pregnancy, and five to six times the levels in the virgin gland, while in thelectomised glands, prolactin binding had returned to levels seen in virgin females. Prolactin binding in intact glands remained high throughout lactation and had returned to the levels in virgin females by 5 days after weaning, when they were not significantly different from the thelectomised glands.

Oestradiol binding increased in intact mammary glands in early lactation and continued to rise as lactation progressed, falling again to the levels seen in virgin females after weaning. In thelectomised glands there was no rise in oestradiol binding, which remained at the levels seen in late pregnancy.

The observations on binding of prolactin and oestradiol to the intact mammary glands confirm previous results in the rat showing marked elevation of both prolactin⁶⁻⁹ and oestradiol⁹⁻¹³ binding in lactation. The failure of prolactin and oestradiol binding to rise after removal of the young on the day of littering has been previously reported⁹, but in the present experiments it is striking that this occurred in the thelectomised glands of rats which were suckling pups on their intact glands and whose serum levels of prolactin would be markedly elevated for at least 15 days⁹. Oestrogen binding to rat mammary tissue is unaffected by ovariectomy¹¹, but increased by prolactin¹⁴. Prolactin binding is increased by prolactin itself and by oestrogen, acting at least in part by stimulating plasma prolactin levels (see ref. 6 for review).

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Table 2 Specific binding of ^3H -oestradiol to intact and operated mammary glands of unilaterally thelectomised rats

Rats	^3H -oestradiol bound (fmol per mg cytosol protein*)		Significance
	Intact mammary gland	Thelectomised mammary gland	
Virgin ($n=4$)	10.8 ± 5.1	9.25 ± 4.75	NS
Early pregnancy (1–11 days) ($n=6$)	6.7 ± 5.4	10.2 ± 10.3	NS
Late pregnancy (12–22 days) ($n=9$)	4.9 ± 4.1	4.3 ± 5.7	NS
Early lactation (2–7 days) ($n=6$)	$8.7 \pm 4.1^\dagger$	4.7 ± 3.8	NS
Late lactation (8–21 days) ($n=7$)	20.9 ± 15.1	5.0 ± 3.7	$P < 0.02$
Weaned (5–12 days) ($n=6$)	8.6 ± 4.1	3.2 ± 3.1	$P < 0.03$

Oestradiol binding was assayed in the cytosol fraction (100,000 g supernatant). Cytosol plus buffer (500 μl) was incubated overnight (5°C) with saturating amounts (2 pmol) of [$6,7\text{-}^3\text{H}$]oestradiol-17 β (44 Ci mol $^{-1}$, Radiochemical Centre, Amersham) in the absence and presence of unlabelled oestradiol (2 nmol). Unbound steroid was removed by incubation at 0°C for 15 min with dextran-coated charcoal (1 ml 0.5% activated charcoal, Sigma, 0.05% dextran T80, Pharmacia). Charcoal was precipitated by centrifugation (1,500g for 10 min) and the supernatants decanted into 10 ml Instagel scintillation phosphor (Packard) and the radioactivity determined using a Packard TriCarb B2450 liquid scintillation counter, with automated external quench correction. The binding was specific for oestrogens being displaced by oestradiol and diethylstilboestrol, but not by testosterone, dexamethasone, cortisol or progesterone. Values are means \pm s.d.; n = no. of observations; NS, not significant, $P > 0.05$.

* Protein measured by a modified Lowry method¹⁸.

$^\dagger P = 0.03$ versus mammary gland in late pregnancy.

When teats are blocked, ligated or removed, milk accumulates in the mammary gland. Early changes depend on whether the litter remains with the mother. If the litter is removed, then even in intact glands milk accumulates and mammary blood flow falls within 8 h as a result of reduced cardiac output and a reduced proportion of cardiac output being taken by the mammary gland¹⁵. If, as in the present experiments, suckling continues but milk removal is prevented, then even more milk collects in the tissue but the blood flow does not fall¹⁵. However, by 36–48 h capillary closure occurs in the mammary gland whether suckling occurs or not⁵, and there is marked reduction in metabolic activity^{1–3}. The collapse of the capillary bed can be seen by blanching of the tissue and was further demonstrated by Silver⁵ by failure of the gland to give a milk ejection response to oxytocin administered intravenously, whereas a response could still be obtained if oxytocin was applied locally to the exposed surface of the gland. Capillaries remained empty beyond 120 h in suckled glands⁵. As involution progresses, the proportion of cardiac output going to the mammary gland falls, apparently regulated locally by depressed mammary activity^{15,16}.

Our results indicate the importance of blood supply and rate of delivery of hormones to the tissue in the control of receptor levels. This could be of considerable importance in the regulation of receptor levels in breast cancer tissue, since angiogenesis, blood vessel development, is stimulated by neoplastic cells¹⁷.

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Novel expression-linked copies of the genes for variant surface antigens in trypanosomes

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Pathogenic African trypanosomes evade the immune system of their mammalian hosts by the sequential expression of alternative cell-surface glycoproteins (reviewed in refs 1, 2). Variant surface glycoproteins (VSGs) purified³ from cloned variants of *Trypanosoma brucei* have similar molecular weights (about 60,000), but differ in amino acid composition³, N-terminal amino acid sequence⁴ and C-terminal structure⁵. We have cloned DNA complementary to the messenger RNAs for four immunologically distinct VSGs⁶ and hybridised these complementary DNAs (cDNAs) with restriction digests of *T. brucei* nuclear DNA, fractionated by gel electrophoresis and transferred to nitrocellulose strips. Each cDNA recognises a unique set of fragments and this basic set is present unaltered in the nuclear DNAs from the four variants. In addition, each probe recognises an extra fragment only in nuclear DNA isolated from cells expressing the VSG corresponding to the cDNA probe. We infer that activation of a VSG gene involves the production of an expression-linked copy of that gene.

The cDNA clones used in our experiments were originally identified⁶ on the basis of their ability to hybridise only to poly(A)⁺ RNA from the homologous variant and this specificity is further documented in Fig. 1. Total poly(A)⁺ RNA from all four variants of *T. brucei*, strain 427, was size-fractionated by agarose gel electrophoresis (Fig. 1a), covalently linked to paper⁷ and then hybridised with one of the four cloned cDNAs. Figure 1b shows that cDNA of variant 117 only hybridises with homologous RNA; the same was found with the other three cDNAs (not shown). The mobility of the RNA species that hybridise is variant dependent (Fig. 1c), the calculated size varying from 2,250 nucleotides (variant 117) to 1,950 nucleotides (variant 221)⁶. This variation correlates with the apparent molecular weights of the corresponding pre-VSGs, deduced from their mobilities in SDS polyacrylamide gels. These decrease in the same order, 117 being 62,000, 221 being 52,000 (ref. 6).

The genes corresponding to these VSG-specific cDNAs were analysed by the Southern blotting technique⁸. Nuclear DNA from one *T. brucei* variant was digested with various restriction endonucleases, size-fractionated by agarose gel electrophoresis, blotted on to nitrocellulose strips and hybridised with each of the four cDNAs. Figure 2 shows results with three of these. Each

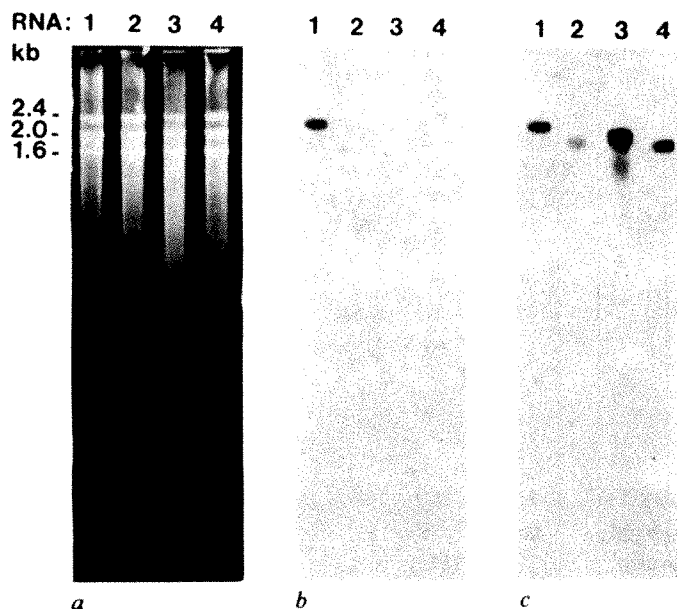


Fig. 1 Hybridisation of cloned cDNA with poly(A)⁺ RNA from the homologous variant and other variants. Glyoxal-treated poly(A)⁺ RNA (2 µg) from each of the four trypanosome variants was electrophoresed through a 1.75% agarose gel in Tris-borate buffer¹⁶. After transfer of the RNA to diazobenzoyloxymethyl paper⁷, the filters were hybridised with nick-translated recombinant plasmid DNA containing sequences complementary to VSG mRNA⁶. *a*, Photograph of the ethidium-stained gel after electrophoresis; lane 1, variant 117 RNA; lane 2, 118 RNA; lane 3, 121 RNA; lane 4, 221 RNA. The large rRNA contains an internal break and yields two bands. *b*, Autoradiogram of the RNA in *a* after transfer to diazobenzoyloxymethyl paper and hybridisation with a recombinant plasmid containing DNA complementary to VSG 117 mRNA (T-cV117-1). *c*, As *b*, but each RNA hybridised with the homologous cDNA probe; lane 1, T-cV117-1; lane 2, T-cV118-2; lane 3, T-cV121-3; lane 4, T-cV221-12. (Figure assembled from different replica gels.) The recombinant plasmids were made by inserting duplex DNA complementary to VSG mRNA into the *Pst*I site of plasmid pBR322 by the dG-dC tailing technique⁶. The sizes of the inserts are (in base pairs): T-cV117-1, 820; T-cV118-2, 1,500; T-cV121-3, 800; T-cV221-12, 1,200. See ref. 6 for isolation of poly(A)⁺ RNA, plasmid isolation, ³²P labelling of DNA by nick-translation, gel handling and other evidence that the cDNA inserts of these four plasmids have no sequence homology under standard hybridisation conditions.

cDNA hybridises to a different set of bands, confirming that each probe 'sees' different genes, as anticipated from Fig. 1. The 118 and 121 probes hybridise mainly to one or two bands in each digest, indicating that they primarily recognise one gene. At long exposures other faint bands become visible, suggesting that the probes cross-hybridise to other genes that show partial cross-homology with the 118 and 121 genes. The 117 cDNA probe hybridises to many different bands in all digests. We attribute this to extensive cross-hybridisation to a family of related VSG genes, because more stringent washing of the hybrids (in 0.1 × SSC at 60 °C) removes the label from all but a few of these bands (not shown). An even more complex pattern has been found with the 221 cDNA (not shown).

There are no differences between the *Hap*II and *Msp*I digests for any of the probes. Both enzymes recognise the sequence CCGG, but cleavage by *Hap*II is prevented by methylation of the C in the CpG doublet, whereas cleavage by *Msp*I is not⁹. Partial methylation of C in the CpG sequence, therefore, does not complicate the digests shown here.

To determine whether expression of a VSG gene results in sequence alterations in or around that gene, we compared the

hybridisation of each cDNA probe with homologous and heterologous nuclear DNA digests. Figure 3 shows results with the 118 cDNA probe. In each digest this probe sees one DNA fragment more in the homologous 118 nuclear DNA than in the heterologous 117 DNA. We attribute the fragments common to the 117 and 118 DNA digests to a basic copy of the 118 VSG gene, present in both variants. The results of double digestion experiments with two restriction enzymes fit a *Pst*I × *Eco*RI × *Bam*HI × *Hind*III map of this basic copy which accounts for all

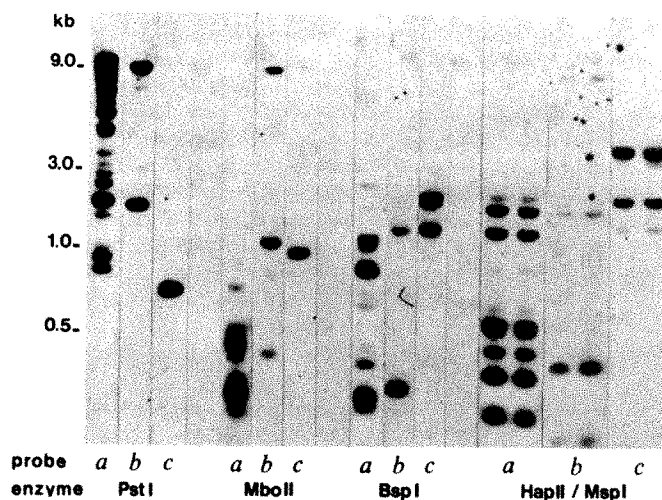


Fig. 2 Autoradiogram of variant-specific cDNA probes hybridised to restriction enzyme digests of nuclear DNA from *T. brucei* variant 118. Nuclear DNA was isolated by a modification of the kinetoplast DNA isolation procedure¹⁷, digested in standard conditions¹⁷ with the enzyme indicated and electrophoresed through a 0.6% agarose gel¹⁷. The gel was blotted on to nitrocellulose filter strips as described elsewhere⁸ and hybridised with nick-translated DNA from the recombinant plasmids indicated. See Fig. 1 legend for further details. Probes: *a*, T-cV117-1; *b*, T-cV118-2; *c*, T-cV121-3.

fragments seen in blots without invoking intervening sequences or the presence of more than one basic 118 VSG gene. In each digest shown in Fig. 3 the 118 cDNA probe hybridises to one extra band in the homologous 118 nuclear DNA, not present in the 117 nuclear DNA. An extra band was also found in the *Mbo*II, *Hap*II and *Msp*I digests (not shown). We attribute these extra bands to an additional expression-linked copy of the 118 VSG gene only present in 118 nuclear DNA.

Analogous results were obtained with the 117 and 121 cDNA probes. Figure 4 shows that the fragments hybridising with the 117 cDNA are identical except for an extra band in the *Msp*I digest of the homologous 117 nuclear DNA. Extra bands only in homologous DNA were also observed in the *Hap*II, *Eco*RI and *Pst*I digests (not shown), but not in *Bsp*I (Fig. 4) and *Mbo*II digests (not shown). The fact that an extra band does not appear in all digests of homologous DNA is not surprising. If the sequences around a gene change, this will only result in altered fragments in blots if the enzyme used cuts far enough from the gene segment recognised by the probe to include the altered sequence. The results with the 121 cDNA are more complex. In this case extra bands are observed in both the 121 and the 221 nuclear DNAs, but the extra bands are not identical in size. It is of interest that variant 221 has arisen during *in vitro* culture from variant 52, which is immunologically identical to variant 121 (ref. 10). The extra band in the 221 digest could, therefore, represent an inactivated form of the expression-linked copy of the 121 gene, but this is only one of several possibilities.

Analysis of several blots like those shown in Fig. 3 has yielded no reproducible differences in the relative intensities of the

[illegible]

Fig. 3 Hybridisation of DNA complementary to VSG 118 mRNA to nuclear DNA digests of variants 117 and 118. The restriction digests were electrophoresed through a 0.7% agarose gel. Further conditions as in Fig. 2. The p marks persistent partial *Hind*III digestion products, absent in other experiments. Lane 1, nDNA 117; lane 2, nDNA 118; P, *Pst*I; B, *Bam*HI; H, *Hind*III; E, *Eco*RI.

To account for the results of the blotting experiments, the formation of the expression-linked copy must be associated by a reshuffling of DNA sequences which alters the neighbouring sequences. This reshuffling could either move the gene into a new (expression) site¹²⁻¹⁴, as in the cassette mechanism for mating-type switching in yeast¹⁴, or an insertion sequence could be put next to the extra copy¹⁵. Whatever the mechanism, the sequences flanking the expression-linked copy of the VSG 118 gene are unusual in that at one side no *Pst*I, *Hind*III, *Bam*HI or *Eco*RI site is present within 8 kilobase pairs of the gene. It is interesting that large extra bands have also been observed when the 117 cDNA is hybridised with 117 nuclear DNA cleaved with *Msp*I (Fig. 4), *Pst*I or *Eco*RI. The flanking sequence, may, therefore, be similar whatever alternative gene is being expressed. The simplest interpretation of our results is that the expression-linked copy is the one transcribed and that the novel flanking sequences are responsible for this; however, this remains to be confirmed.

The recombination event that we expect to be responsible for the positioning of the extra copy in the postulated expression site could easily be accompanied by further recombination events that increase sequence diversity in the VSG genes. There is no

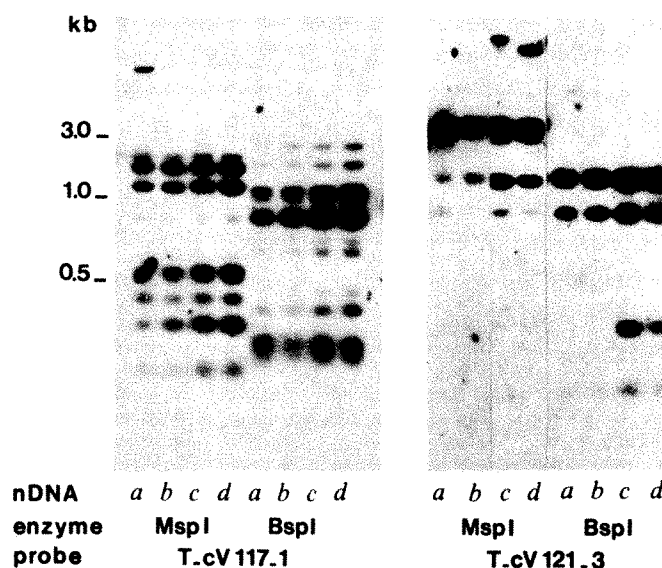


Fig. 4 Autoradiogram showing hybridisation of VSG-specific cDNA probes with homologous and heterologous nuclear DNAs. Nuclear DNA from the four variants indicated was electrophoresed through a 1.2% agarose gel; see Fig. 2 legend for further details. nDNA: a, 117; b, 118; c, 121; d, 221.

evidence for this either in previous work on VSGs (refs 2-5 and G.A.M.C., unpublished) and their genes⁶, or in our present experiments, as the expression-linked copy is not recognised by the heterologous probes tested. It seems likely, therefore, that antigenic diversity in trypanosomes is genomic and finite and not created by a clever reassortment of a limited number of DNA sequences to yield an unlimited number of VSG genes. It is possible, however, that the novel flanking sequence in the expression site provides not only a promotor but also the 5'-leader sequence and signal peptide (if present) for each VSG. This can be tested when genomic clones are available; work is in progress to obtain them.

Williams and coworkers¹⁸ have also observed rearrangement of DNA associated with expression of another line of *T. brucei*. In their case, however, there is no clear conservation of bands associated with a basic copy and, moreover, there are also alterations in another clone apparently not associated with expression. We cannot account for this crucial difference from our results.

We thank Miss J. Van den Burg and Mr M. J. Hillebrand for assistance and Professor C. Weissmann for providing plasmid DNAs. This work was supported in part by a grant to P.B. from BION (ZWO) and a Research Training Grant to A.C.C.F. from WHO.

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Spiders

E.O. Wilson: Let me remind you that people everywhere, a large percent of the population, at a very early age have already developed a deep horror at the sight of snakes or spiders with nothing more than gentle nudging from their parents, if that. Yet, in spite of the fact that parents constantly reinforce their children against going near electric sockets, automobiles, knives and the like, phobias against such objects rarely develop.

Marvin Harris: Let's go back again to the possibility that these phobias are genetically programmed — which I'm willing to grant. The overwhelming bulk of the socially conditioned response repertoires of different human societies consists, by your own admission, of culturally determined rather than genetically determined traits. Then it seems to me that when one offers a cogent culturological explanation of these phobias, one has to be considered that this explanation is not offered in isolation.

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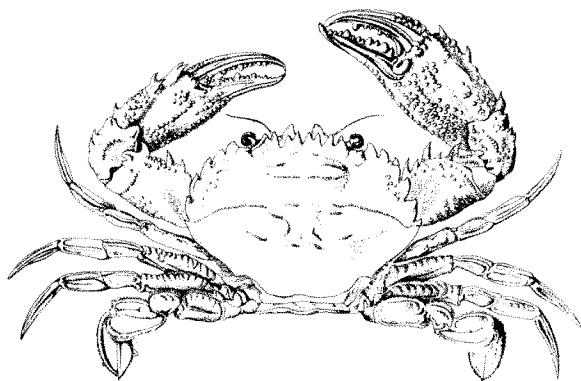
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US COLLEGE TEXTBOOK SUPPLEMENT

Bringing physics to life

Daniel Zwanziger

Physics (Formerly Life Science Physics). By Joseph W. Kane and Morton M. Sternheim. Pp.664. (Wiley: New York and Chichester, UK, 1978.) £11. *General with Bioscience Essays*. By Jerry B. Marion. Pp.539 plus index. (Wiley: New York and Chichester, UK, 1979.) \$18.95. *Physics for Applied Biologists*. By N.C. Hilyard and H.C. Biggin. Pp.223. (University Park Press: Baltimore, 1977.) \$13.75. *Intermediate Physics for Medicine and Biology*. By Russell K. Hobbie. Pp.557. (Wiley: New York and Chichester, UK, 1978.) \$23.95.

It would be hard to overestimate the degree of penetration of the concepts of physics into biology. On the one hand, the conceptual barrier between the living and the non-living world has continually eroded as our detailed knowledge of biological processes has increased. On the other hand, the introduction of new instruments and techniques resulting from advances in physics has in many cases opened up whole new areas of biology. Today's laboratory investigators use sophisticated instruments to study the function of an organ, cell or macromolecule which is ultimately understood by means of a simple physical concept such as diffusion, electrical conduction or tunnelling through a potential barrier. The two principal themes are entropy and energy which distinguish spontaneously occurring processes, namely those accompanied by an increase of entropy from active or pumped processes where entropy decreases at a cost of energy. The detailed knowledge of living things achieved by present day biologists is a triumph of the mechanistic world view in which, to be sure, quantum mechanics plays an important role. It is well recorded in Jacques Monod's broadly painted overview of biology *Le Hasard et la Nécessité* (Editions du Seuil, Paris, 1964).

It is fortunate that a number of texts have appeared to meet the need of the biologist to learn physics. Outstanding among these is *Physics with Illustrative Examples from Medicine and Biology* (Vol. 1: Mechanics by G.B. Benedek and

F.M.H. Villars; Vol. 2: Statistical Physics by F.M.H. Villars and G.B. Benedek; Vol. 3: Electricity and Magnetism by G.B. Benedek and F.M.H. Villars, Addison-Wesley: London and Reading, Massachusetts, 1973, 1974, 1979). These volumes are a milestone of pedagogy. They successfully integrate the history of the subject and the analysis of key experiments into its logical exposition. (This history reveals, incidentally, the corresponding debt of physicists to physiologists such as Volta, Mayer and Helmholtz, who in fact have made many of the important discoveries of physics.) These volumes provide a wealth of anatomical and physiological applications worked out in beautiful and patient detail. In the opinion of this reviewer they provide the best introduction available to statistical physics as pure physics, entirely apart from biological applications. Any text on the subject must be measured against the standard which they set. It seems, however, that the blinding light of truth cannot be revealed all at once to the uninitiate, and

that the content of these volumes is too rich for a first introduction to physics for those whose main interest is in the life sciences, and who generally take only two semesters of college physics. However, they make a wonderful text for an intensive three-semester introductory course in physics if the students are reasonably well motivated.

A number of texts have been published in recent years for a two-semester introductory course in physics with applications in the life sciences. Of those which this reviewer has examined, perhaps a dozen in all, the best by far, in his opinion, is *Physics (Formerly Life Science Physics)* by Kane and Sternheim. Most of these texts, written by physicists for the pre-medical student market, are basically standard physics texts with occasional applications of biology. The conspicuous virtue of the text by Kane and Sternheim, which distinguishes it from its competitors, is the breadth of coverage of the many subfields of physics and the remarkable completeness of the quantitative applications to the life

Sir John Kendrew and his model of myoglobin — the first protein to yield its structure to the physics of X-ray diffraction.



sciences. The latter, taken together, could constitute the core of an introductory course in biomechanics and biophysics. The authors achieve this without sacrificing the derivations which are the meat of any course in physics, by clear and concise writing which allows them to present more on each page than the other texts. The wealth of material may be used selectively to advantage emphasising either the basic physics or the life science applications. Derivations which use calculus are segregated to the end of each chapter. There are abundant homework problems of graded levels of difficulty.

General Physics with Bioscience Essays by Jerry B. Marion is a text for a one-year non-calculus terminal course in physics. Though only slightly shorter than *Physics (Formerly Life Science Physics)* by Kane and Sternheim, it contains fewer derivations and fewer and less detailed life science applications, with no corresponding gain in clarity.

Physics for Applied Biologists by N.C. Hilyard and H.C. Biggin is a useful compendium of formulae and diagrams of instruments. However, to this reviewer's mind it is not really a physics text at all because derivations, as examples of physical reasoning, are almost entirely lacking. If physics-for-biologists texts become distinguished from pure physics texts by the absence of reasoning, they will

contribute to the division of the house of science instead of its unity. The phenomenon is before us in the statistics-for-social-scientists texts, generally written by social scientists instead of mathematicians, in which the subject is deprived of its logical structure and reduced to a verbose hodge-podge and unrelated formulae, because it is assumed that the student is incapable of understanding a logical derivation. The vicious circle is fatally closed when the student does indeed conclude that he is incapable of understanding statistics.

Intermediate Physics for Medicine and Biology by Russell K. Hobbie brings together material not readily available elsewhere. It is the only text on the subject, which this reviewer has seen, in which a knowledge of calculus and a previous acquaintance with physics are assumed. The author was motivated by the sophisticated level of physics applications in medical school courses and the poor preparation in physics prevalent among medical students. He has an ambitious aim, namely a logically complete though concise presentation of physics, without reliance on "it can be shown", though the main body of the text is devoted to applications in the life sciences. The result is successful. For example, the brief introduction to statistical physics is applied effectively in subsequent chapters to the phenomenon of

osmotic pressure and transport through membranes. Similarly, sufficient electricity and circuit theory are presented for a cogent exposition of the Hodgkin-Huxley model of nerve conduction and the electrocardiogram. The quantum effect in dark-adapted vision, discovered by Hecht, Schlaier and Pirenne, is clearly explained. It would be helpful if, in a new edition, more anatomically correct drawings accompanied the physicist's schematic or block diagrams. This text is both competitive with and complementary to the volumes by Benedek and Villars which I praised above. There is considerable overlap of subject matter, but Hobbie assumes greater background on the part of the student and thus can be more concise. His book would make an excellent text for an upper division undergraduate course. Such a course could profitably be introduced in many universities. For physics students it would be a valuable introduction to applications in the life sciences which may offer them interesting career opportunities. It would be invaluable for graduate students preparing for research in the life sciences, where the concepts of physics have assumed overwhelming importance. □

Daniel Zwanziger is Professor of Physics at New York University.

Physics starts here; or here?

David J. Miller

Concise Dictionary of Physics. By J. Thewlis. Pp.366. (Pergamon: Oxford and New York, 1979.) £20.00. *Introductory Physics.* By M.L. Warren. Pp.683. (Freeman: San Francisco and Reading, UK, 1979.) £20.50. *Physics.* By Jay Orear. Pp.752. (Collier-Macmillan: London, 1979.) £13.50.

SHOULD physics be taught as a body of facts or as a system of ideas? These three books at introductory college level reflect the most divergent of views.

The *Concise Dictionary of Physics* claims in its preface to be intended not only for students but also for teachers, engineers, journalists and for the interested layman. It is surely a useful compilation; by no means comprehensive but amazingly good for 370 pages, and admirably concise. In sampling an arbitrary list of topics which I had decided in advance it ought to contain only one was missing — Clebsch-Gordan coefficient. There are few inaccuracies, though baryons are no longer those "fermions with mass greater than or equal

to that of the nucleon". The chief criticism to be made is not of the dictionary's accuracy but of its relation to the rest of the world of physics. Concepts here are "formulated, sprawling on a pin", lifeless and unconnected with the world in which they live. When so much trouble has been taken to write the definitions it seems perverse not to offer references to textbooks, or bibliography. I would like to know what a "quaternion" is, for instance. Where do I go next after reading that it is "an operator, in a system of vector analysis invented by Hamilton, which changes one vector into another by rotation accompanied by a change of magnitude"?

Jay Orear's *Physics*, an introductory textbook for science and engineering students, goes to the other extreme. He begins by saying that the goal of physics is "to seek out and understand the basic laws of nature upon which all physical phenomena depend." The book reflects this statement, with a methodical progression from mechanics to special relativity, followed by heat and thermodynamics, then electricity and magnetism, waves and optics, quantum physics, atoms, molecules, solids, nuclei, astrophysics and particles. It is assumed that students will learn calculus in parallel with this course, so he is able to present the "basic laws of nature" in their

mathematical form. This is not a book for biologists or medical students, but physics students will want a more complete account of each of the subjects introduced here. In 752 spaciouly laid out pages there is no room for detailed experimental justification of many of the assertions made and there are surprisingly few illustrations. Its use will probably be in those universities where an introductory physical-sciences class is given high powered courses in mathematics, physics and chemistry before separating into specialised groups. Compared with a rival such as the three-volume *Fundamental University Physics* by Alonso and Finn (Addison-Wesley: Reading, Massachusetts and London, 1967), this book has the advantages of being up to date and short enough for the logical structure of the whole subject to be clear. There are twenty or thirty problems at the end of each of the thirty chapters, with answers to the odd-numbered problems at the back. A bibliography of more specialised texts would have been useful.

Introductory Physics by Mashuri L. Warren is aimed at biologists and medical students. My only objection to it is that its organisation is sometimes more like that of Thewlis' dictionary than can really be justified in a textbook. Where Orear never loses sight of physics as a unified system of ideas, Warren treats it as a body of often

unconnected facts. A strange example is the treatment of the equivalence of mass and energy, attributed to Einstein and discussed in some detail, but in no way related to the subsequent chapter on the special theory of relativity. But, having grumbled a little, let me say what a useful book it is. It crams an enormous amount into 683 double-columned pages, with line drawings or photographs of equipment on every page. Because the student is assumed weak in mathematics, most results quoted

are backed up by generous experimental — even anecdotal — detail, as well as some basic mathematical exposition. Physics for its own sake gets little space — particle physics hardly figures at all — but there is a chapter on electric shocks and electrical safety which taught me a great deal that I should have known before; and the chapters on optics and hydrodynamics are sensible and detailed. There are twenty or thirty simple problems at the end of each chapter, with answers to the odd numbers

at the back. The bibliography for each chapter is eclectic — not many specialised textbooks but a range of historical material, memoirs of scientists and *Scientific American* articles. There are appendices on basic mathematics, physical constants and units, ‘well known functions’ and problem-solving with calculators. □

David J. Miller is a Lecturer in the Department of Physics and Astronomy, University College London, UK.

Remote sensing of the environment

R.N. Colwell

Introduction to Remote Sensing of the Environment. Edited by Benjamin F. Richason Jr. Pp.496. (Kendal/Hunt: Dubuque, Iowa, 1978.) \$27.00. *Laboratory Manual for Introduction to Remote Sensing of the Environment.* Edited by Benjamin F. Richason Jr. Pp.237. (Kendal/Hunt: Dubuque, Iowa, 1978.) \$8.95.

THESE two books are companion volumes prepared by 24 contributors under sponsorship of the National Council for Geographic Education and published in the Council's Pacesetter Series.

The first chapter of the textbook provides a stage-setting overview. The second chapter describes the nature of electromagnetic energy and of basic matter/energy relationships. The next eight chapters describe various remote sensing devices and aerospace image acquisition systems; included are illustrations of the interpretation of black-and-white, colour, and colour infrared photographs, and of imagery acquired by thermal infrared scanners, multispectral scanners and microwave sensors. The next nine chapters deal with specific remote sensing applications including the analysis of landforms, rural cultural landscapes, agriculture, forestry, urban and industrial areas and weather phenomena; cartographic and regional planning applications are also included. The concluding chapter constitutes both a summary and a look to the future. The book's four appendices list sources from which remote sensing imagery and data tapes can be obtained, provide reference materials for beginning students, and present colour reproductions of various types of imagery. They are followed by biographical information about the contributing authors and a brief subject index. A much needed glossary is not included. The laboratory manual closely parallels the textbook, with appropriate emphasis on applications.

The scope of these two volumes is

excellent and the concept of multiple authorship is commendable. The quality with which the image examples are reproduced ranges from good to excellent and most of the writing is highly articulate. Closer study of the textbook suggests, however, that its chapters comprise more a compendium of separate treatises than a well integrated introduction to remote sensing. One manifestation of this is a lack of standardisation of terminology. For example, is ‘remote sensing’ (the most fundamental of terms) limited to the mere ‘imaging of features’, as defined in the Preface? Does it include the obtaining of digital records, as implied later in the text? In addition to data acquisition, is data analysis encompassed by this term? If so, does the analysis encompass work done not only by humans but also by machines? With respect to balance: (1) symptomatically, the chapter on agriculture planning is given roughly four times this emphasis in both respects; (2) of the 24 contributors, no less than 20 appear to be geographers; (3) more than half of the 24 colour plates pertain to features in the Great Lakes area; and (4) while the captions for a few of these colour plates are quite complete and are well cross-referenced to the two books, the captions for others indicate only the image type and/or geographic location.

Superficially, the laboratory manual is

very impressive, well illustrated and well matched to the supporting textbook. On a closer look, several of the exercises appear decidedly superficial. Also, the limited use of aerial photo stereograms gives the erroneous impression that three-dimensional perception and interpretation are of little importance.

There are notable strengths to offset these deficiencies. For example, Chapter 2 provides a masterful disclosure of some very important but highly complex material at a level that can be readily comprehended by most beginning students. Similarly, Chapter 16, dealing with regional planning, is clearly expressed, reasonably comprehensive, well balanced and well illustrated.

In summary, these two books represent a commendable effort to satisfy a long-standing need, that of providing the beginning student with a reasonably comprehensive remote sensing textbook and laboratory manual that might be digested in a single four- or five-unit course at the college or university level. A more carefully edited and better balanced second edition would far better satisfy this need. □

R.N. Colwell teaches courses in photo interpretation and remote sensing at the University of California, Davis, California.

Practical organic chemistry

J.E. Girard

Practical Organic Chemistry: A Basic Course. By V.V. Nekrasov. Pp.410. (MIR Publishers: Moscow, 1978.) \$7.80.

THIS fifth edition of a popular Russian text has been translated into English by Alexandar Rosinkin. What is not evident from the title of this book is that it is a laboratory text for students at vocational or technical school or for non-chemistry university students. As the author states in the introduction, this text is intended to be ‘only a practical manual, and it cannot rival the textbook of organic chemistry.

The theory is given in a limited scope’.

For the most part, the chapters are arranged according to functional groups. Within chapters are experiments dealing with qualitative organic analysis and simple synthesis. The latter chapters deal with polymer reactions, precipitation and denaturing proteins, hydrolysis of fats, preparation of mustard oils and reactions of carbohydrates.

Although this is a very thorough treatment, it is somewhat out of date. The translator has unfortunately chosen to use words or phrases that are not commonly used. This may cause beginning students undue confusion. □

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Physical chemistry texts

David S. Kliger

A Textbook of Physical Chemistry. Second edition. By Arthur W. Adamson. Pp.953. (Academic: New York, 1979.) \$22.95. *Physical Chemistry.* Fifth edition. By Robert A. Alberty and Farrington Daniels. Pp.682. (Wiley: New York, 1979.) \$19.50. *Physical Chemistry: A Step-by-Step Approach.* By Marwin K. Kemp. Pp.1034. (Marcel Dekker: New York, 1979.) \$23.75.

In *A Textbook of Physical Chemistry* Adamson has put some new twists into the traditional format of physical chemistry texts. The ordering of topics, for instance, is significantly altered; a chapter on additive physical properties of matter appears near the beginning of the book. This chapter covers absorption of light, molar refraction, molar polarisation, and dipole moments. These topics would normally be found much later, after discussions of molecular structure. Another novelty is the placement of statistical mechanics in this text. Rather than devote a separate chapter to this topic Adamson includes discussions of statistical mechanics in chapters on thermodynamics. This makes the link between statistical mechanics and thermodynamics more apparent than in many texts.

Other non-standard features of this text include 'Commentary and Notes' and 'Special Topics' sections at the end of each chapter. These contain expansions of topics covered in the chapter, discussions of historical points of interest, and brief discussions of related topics. Unfortunately, some of these sections discuss material much more advanced than material covered in the related chapter. Also, many of the 'Special Topics' are topics normally discussed in physical chemistry texts. Here they are merely added at the end of chapters rather than integrated into the main text.

Overall, this book is a rigorous, rather sophisticated text that would make a fine reference source. It has a fine index and many good figures which help clarify the text. As a book to introduce students to the subject of physical chemistry, however, it seems too sophisticated. Many discussions are too advanced and the ordering of topics sometimes makes the material difficult to understand. There are many problems for students to solve, separated into sections of straightforward exercises, demanding problems, and special topics problems. These are fine learning tools for students. For all but the most sophisticated student, however, the main text will probably be difficult to follow.

Physical Chemistry, by R.A. Alberty and F. Daniels, is a rather traditional

textbook for a year-long physical chemistry course. It presents little history behind the subject areas or motivation for studying them. Instead, the emphasis seems to be on presenting formulae to be used in a physical chemistry course. This may be reasonable for use in a course based on a lecture format where the instructor provides background and motivation. This approach leads, however, to a rather dry text. Consistent with this format of a text to supplement lectures, the book presents many problems and answers to about half of them. This is probably the strongest feature of the book from the instructor viewpoint.

Treatments of various topics in the book vary greatly. Many topics are presented too tersely, making it seem that learning facts and formulae is more important than understanding underlying principles. This seems particularly true of the coverage of quantum theory and, to a lesser extent, of thermodynamics. Later sections covering Statistical Mechanics and Kinetics, however, are quite well done.

Some topics covered in this book, such as equilibrium for biochemical reactions, macromolecules, and surface thermodynamics, are not found in most textbooks of physical chemistry. They add a very nice supplement to the traditional topics found in most texts. On the other hand, these chapters again tend to emphasise facts rather than present a general overview of these special topics.

In all, this book would be useful in courses where lectures provide the primary source of fundamental material. It does not, however, stand on its own as a primary learning tool for physical chemistry.

Physical Chemistry: A Step-by-Step Approach, by M.K. Kemp, differs from

other physical chemistry texts in that it is designed for use in a self paced (sometimes called 'Keller Plan' or personalised system of instruction) course. In such a course there are often no lectures and the book becomes the primary source of information for students. Thus, the book must be very clear. This book is just that.

Each topic in the book is introduced with a statement of the importance of understanding the topic as well as a list of learning objectives the student must keep in mind. After discussion of each topic there are problems, with detailed solutions given in an appendix, and a self test. A detailed table of contents and a list of symbols and definitions also precede each topic to aid the student.

This book has very few weak points. There are not as many problems as one might wish and the introduction to quantum theory is weak. However, later sections on atomic structure and spectroscopy are excellent. Most topics are introduced at a very elementary level and progress through to a rather high level of sophistication. Treatments of real gases, colloid and surface chemistry, and polymers are particularly thorough.

In all, this is an outstanding text for physical chemistry courses using a self paced format or a standard lecture format. *Physical Chemistry*, by P.W. Atkins (Freeman: San Francisco, 1978), is the only other physical chemistry text this reviewer has seen which matches this book in clarity of presentation. □

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Physical chemistry for the life sciences

R.M. Harris

Physical Chemistry with Applications to the Life Sciences. By David Eisenberg and Donald Crothers. Pp.868. (Benjamin Cummings: Menlo Park, California, 1979.) \$21.95.

THIS book surpasses all other life science-orientated physical chemistry texts I have examined in both the extent and depth of its coverage of traditional physical chemistry topics. Thermodynamics, solutions, electrochemistry, statistical mechanics and X-ray structure analysis are all presented at about the same conceptual and mathematical level as found in standard 'for chemists' texts, such as the most recent edition of *Physical Chemistry* by Moore (Prentice-Hall: Englewood

Cliffs, New Jersey, 1972). This is not to say it could replace a text like Moore's in a traditional two-semester 'for chemists' course. Its treatment of atomic structure and chemical bonding is neither extensive nor rigorous enough, and it has little to say about gas properties, kinetic molecular theory or reactions in the gas phase.

This text also has much to offer in the way of biological applications. Along with the usual topics (enzyme kinetics, transport properties, membrane equilibria, bioenergetics) it provides a more extensive treatment of macromolecular biophysics, including DNA characterisation, than any of the competing texts I know of, and it contains a lengthy qualitative section on the application of symmetry to the description of biological systems. Another attractive feature is that many of the mathematical results derived in the text are accompanied by a verbal description of their physical significance, and in most instances the explanations successfully illuminate the equations.

I was disappointed to find that several important biochemical topics normally included in a text of this kind were omitted by Eisenberg and Crothers. Aside from a paragraph on transition-state analogues, there is no discussion of enzyme inhibition, and there is nothing in the way of photochemistry or photobiology apart from a passing reference to photosynthesis. I also feel that the text needs more illustrations, and many that are present are too small or too sketchy. In

reading the sections on membrane phenomena, for example, I missed not seeing a picture of the modern model of a biological membrane, and the illustration accompanying the discussion of the hydrophobic effect did not help me at all in envisioning 'icebergs'. My most serious concern is that some of the major topics are developed in an unnecessarily formal manner. The reader has to labour through an entire chapter on principles of spectroscopy, and several sections of the

ensuing chapter, before encountering illustrative chemical examples.

Overall, the book's good points outweigh its shortcomings. You will want to give it serious consideration if you are seeking a text that offers life science students a solid foundation in physical chemistry. □

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Biology from a human angle

M. Edidin

Inquiry into Life. Second edition. By Sylvia S. Mader. Pp.771 plus appendix, glossary, index. (Wm. Brown: Dubuque, Iowa, 1979.) \$15.95. *Biology. Human Perspectives*. By Charles Kingsley Levy. Pp.562. (Goodyear: Santa Monica, California, 1979.) \$17.95. *Man, Nature and Society. An Introduction to Biology*. Second edition. By E. Peter Volpe. Pp.662. (Wm. Brown: Dubuque, Iowa, 1979.) \$15.95. *Biology. A Human Approach*. Second edition. By I.W. and V.G. Sherman. Pp.636. (Oxford University Press: 1979.) \$16.95.

ALL four of these books aim to introduce biology to students beginning a college education. It must be assumed that such students have no background at all and instruction must begin with the most basic and general aspects of biology. The field is notable, even to one who works in it, as short on theories and general organism principles and long on variety. The broad problems of life — maintenance, regulation and reproduction — are approached in a bewildering diversity of forms and treated in many different ways by living organisms. How does an instructor construct an introductory course from such diverse material? The books under discussion do so by concentrating on the biology of a single species, humans, discussing important generalities in terms of human examples and then continuing with discussion of specific problems or processes peculiar to the species and its relatives. Around 98% of all organisms are excluded by this approach.

The reasons for concentrating on the biology of a single species are variously stated by the authors. Mader's book, *Inquiry into Life*, "emphasizes the application of this knowledge to modern concerns . . . biology is truly relevant." *Biology. Human Perspectives* by Levy aims to avoid fragmentation and to con-

centrate on the biology of the most intensively studied organism. He, too, feels that this biology will have the greatest "appeal, relevance and interest . . ." for students, busy enquiring humans that they are. Volpe in *Man, Nature and Society. An Introduction to Biology*, wishes to educate liberally, to present biology so that intelligent use can be made of it by humans who, though not extensively trained in science, can recognise the importance of biology in many social and ethical problems in society and can approach such problems thoughtfully and rationally. The Shermans in *Biology. A Human Approach* similarly hope to present information that will enable students "to understand themselves and the world in which they live." They, too, consider personal relevance and "areas of immediate human concern" important to their presentation. All the books, then, wish to show us biology epitomised in ourselves and as affecting us in the world today.

The four books, though attempting more or less the same feat, do not approach the subject with the same intellectual views, principles of organisation or physical orientation. Also, they do not seem intended for the same students. The intellectual level of the texts varies greatly, as does the emphasis on the material presented.

Before commenting specifically, I ought to note my own prejudices. First, I am not sure that the human organism is so interesting or so dominant that it is worth an entire general biology book. Many humans think otherwise; three of the texts considered here are in second editions. Second, to my mind, the only truly integrating approach to general biology is an evolutionary and genetic approach. Without this, texts fragment into sections not easily connected to one another. The first and second prejudices reinforce each other. Third, I have been teaching a general biology course, using several different texts, over the past eight years. My reactions to new texts are dulled, rather than heightened by the experience.

Of the four books, by far the most interesting, easy to read and best organised was Volpe's. Rather than begin with a discussion of 'life' and its origins, or with a long section on chemistry, Volpe begins

with a discussion of human reproduction, nicely balanced between endocrinology and anatomy. This is followed by an outstanding chapter on the control of fertility in humans which in turn is followed by a clear descriptive discussion of vertebrate development and birth. A clever transition is then made to consideration of the biochemistry needed for modern biology by considering the problem of trans-placental transfer of nutrients. We then return to cell differentiation and development. The section is concluded with seven clearly written chapters on physiology and endocrinology of adults. Two sections follow on genetics, evolution and population genetics. These constitute nearly half the material in the book and take the student from the chemical basis of inheritance via biochemical genetics and simple population genetic studies of selection and evolution, to questions of polygenic inheritance, speciation and adaptive radiation. The concluding section covers other aspects of populations and biological communities. Appendices on simple chemistry and on probability follow the body of the text. Throughout the writing is clear; transitions between subjects are deftly made, and the material is accurately presented. The standard of illustration is high; both colour photographs and coloured full page drawings are used in addition to smaller black and white photographs and drawings. I regret only that this text, like most others in the field, does not supply scales to each picture. Biology deals with structures over at least a millionfold size range. Certainly, beginners ought to be helped to some sense of scale. Volpe's book is directed at intelligent beginners. It is first rate overall and should stimulate both students and teachers using it.

The three other texts considered here are rather more like each other than not. Sherman and Sherman and Levy present material in a traditional manner, emphasising physiology and structures far more than genetics and evolution, though the latter subjects do make an appearance. The Shermans' book is clearly written, though the use of boldface to introduce important words and phases makes the text rather shout at the reader. I concede that students in this country often apply liberal amounts of yellow highlighting to achieve

the same result, but I would rather shout myself than be shouted at. The quality of drawings and photos is not as high in this book as in Volpe's, but the drawings especially are clear. The text begins with a discussion of the origin of life and the methods of science, considers cell architecture, biologically important molecules and cell energetics. The first section closes with a discussion of the chemical basis of heredity and of cell division. A long second section is devoted to development and physiology and this is followed by a shorter section on mendelian and population genetics. Overall, the book is workmanlike, but I did not find it especially exciting. Its design also made it difficult reading. The book is printed largely in shades of blue ink, including the text itself. This is hard on the eye.

Charles Levy's book, the only one of the four making its first appearance, is

similarly organised, but the bulk of the book considers physiology and regulation. Genetics and development are given only two chapters at the end of the text, about 60 pages out of about 550. I do not think that this balance is correct, but those who wish to emphasise human physiology may find the text useful. However, it should be noted that there are a number of errors of fact in both text and drawings. The creation of a text is a difficult job and I think that allowance for errors should be made. Bright students will catch many of them (for example the jump from calories to kilocalories that occurs from pages 57 to 58). Physically, the book is somewhat smaller than the two others already discussed and this cramps the illustrations, which are often deployed in the margins of the text. The audience for this book as for the previous text is the group of average college students. They can learn from both

books through diligent application, but I am not sure how interested they will be in either of these texts.

The last book to consider, by Mader, is directed at a less sophisticated audience than the other three. It is written in a simpler style and readers are talked at, rather than being shown the development of an idea. There is, from my point of view, an excessive catering to students' needs for information on, and concern with, drug action, and with venereal diseases. A large quantity of biological information is contained in this book. The author is trying to deliver it to students who may be preoccupied with matters other than classwork. I am not certain that the approach emphasising 'relevance' to society is the one to use. □

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Primate cytogenetics

Dorothy A. Miller

The Phylogeny of Human Chromosomes. By Héctor N. Seuánez. Pp.189. (Springer: Berlin, Heidelberg and New York, 1979.) Paperback DM38; \$20.90.

CURIOSITY about the origin of man has led to the application to evolutionary problems of a variety of new techniques. *The Phylogeny of Human Chromosomes* by Héctor N. Seuánez deals with information derived from the use of the chromosome banding techniques that were developed during the past ten years.

The initial chapters present a brief overview of human evolution. This is useful as background, but the author fails to point out that chromosome studies can be carried out only on living organisms. Although we might like to know the karyotype of *Homo erectus*, comparisons must be made among species that exist at the present time. This volume, then, deals primarily with comparison of the karyotype of human with those of the great apes — chimpanzee, gorilla and orangutan. One of the results of comparative chromosome studies has been to place the gibbons and siamangs in a family separate from the great apes, with which they were classified at one time.

Brief descriptions of the major chromosome banding techniques are presented. G- and R-banding are used to identify every chromosome in the complement. (Q-banding is used for the same purpose, not just for identifying brilliant variant regions, as the author implies.) G-banded karyotypes of human, gorilla, chimpanzee (two

species) and orangutan are illustrated. Direct comparison of the various species would have been facilitated by presenting more composite karyotypes in which the presumptive homologues are placed side by side. It is unfortunate that the figures in chapter 7, where some of the chromosomes are placed together for comparison, are not arranged as effectively as are those in chapter 6 in which chromosomes of different orangutans are compared.

The most exciting finding in primate cytogenetics has been that every human chromosome has a recognisable counterpart in each of the four species of great apes. (This is in marked contrast to the results obtained in the rat and mouse, which are also evolutionarily related species but in which only a few chromosomes have similar banding patterns.) Some reorganisation of the genetic material has taken place during the evolution of the higher primates and man. There has been one fusion that results in the human having 46 chromosomes compared to 48 in the great apes. The most frequently observed differences between the chromosomes of the four species are the result of pericentric inversions, which involve a break on each side of the centromere of a chromosome, with rejoining of the inverted segment within the same chromosome. In a practical sense this has facilitated the comparative studies because single altered chromosomes are much easier to recognise than multiple reciprocal translocations. There is no evidence that the pericentric inversions which have become fixed in primate evolution have affected the activity of the genes on these chromosomes.

The demonstration that there are chromosomes with similar banding patterns in humans and great apes is of

much more trivial interest because these chromosomes often carry the same genes. Somatic cell hybrid methods have been used to show that one or more genes on almost every human chromosome has a counterpart on the purported ape homologue. In only one or two cases was a particular gene not carried by the chromosomes that had similar banding patterns in the various species. Similar comparisons with rhesus and African green monkey chromosomes show that genes carried by human chromosome number 1 have been associated with a chromosome segment having the same banding pattern for perhaps 35 million years.

The 18 and 28S ribosomal RNA genes are in a rather special class because they are present in hundreds of copies per cell rather than in 2 or at most a few copies per cell, as is true of most genes. Extensive redistribution of the rRNA genes has taken place during evolution. Most primates have these genes concentrated in a single site, but the orangutan has rRNA genes on 9 pairs of acrocentric chromosomes, the chimpanzee and human on 5 pairs (not all the same), and the gorilla on 2 (or 3) pairs.

Detailed comparisons are presented of the results obtained using methods that provide information about heteromorphic chromosome regions, many of which contain highly repetitive DNA sequences. Quinacrine-brilliant regions, for example, are found only in chimpanzee, gorilla, and human, and a quinacrine-brilliant Y is present only in the latter two species. Human satellite DNAs I, III and IV generally anneal to corresponding sequences on fixed metaphase chromosomes of each of the great apes, although to only a small extent in the orangutan. Human satellite II, on the other hand, anneals to gorilla and orangutan chromosomes but

not to those of the chimpanzee. If human satellite II-like sequences are present in the chimpanzee, they must be there in very small amounts, a finding that is supported by the absence of much 5-methylcytosine, a rare base which is abundant in human satellite II.

Dr Seuánez is at his best when describing his own studies with the orangutan, in which he has shown that animals from Borneo and Sumatra differ by a pericentric inversion (perhaps marking incipient species), but that each population is still heterozygous for a different complex

chromosomal rearrangement. In this volume he has included a wealth of information about a broad range of techniques. Students and those interested in primate cytogenetics will find the volume useful because it includes a large number of references, although a single reference list would have been easier to use than will be the separate list presented after each short chapter. General interest readers will probably be disappointed by the book because the broader aspects of phylogeny have been submerged in the sea of minute details. A final chapter drawing together

information derived from the various methods would have been a welcome addition. Most of the illustrations are well designed, but the chromosomes in some of the composite karyotypes are so small that they present little information. Unfortunately there are a large number of typographical errors which distract attention from the message of the text. □

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Basic molecular biology

G.C. Walker

Basic Molecular Biology. By Fred W. Price. Pp.497. (Wiley: New York, 1979.) \$20.50.

BASIC MOLECULAR BIOLOGY by F.W. Price is intended to be a suitable text for freshmen with a background in general biology and chemistry. The book opens with an extremely elementary introduction to basic chemical principles and then is divided into four main sections. The first section, which comprises almost half of the book, discusses primary through to quaternary structures of proteins, and the relationship of structure to function. One chapter provides a particularly clear general introduction to enzymes and others discuss topics such as fibrous protein assemblies and antibodies. The second section covers the structure of biomembranes and biological energy transducers. The third section covers the structures and roles of nucleic acids in the storage and expression of genetic information. A somewhat speculative chapter on chromatin structure is included. The final section is quite broad and considers the organisation of prokaryotic and eukaryotic cells.

In his preface, Price states that his approach is 'structural'. Indeed, almost every topic is introduced by a consideration of the relevant chemical and structural parameters of the molecule or assembly in question. Since the author has also intentionally minimised the attention paid to molecular genetics and protein synthesis, the book is much closer in many respects to a biochemistry text than to books such as *Molecular Biology of the Gene* (Watson; W.A. Benjamin: Menlo Park, California, 1976) or *Molecular Genetics* (Stent and Calendar; Freeman: San Francisco, 1978). In particular, while genetics is treated as a subject in one chapter, very few genetic experiments or

approaches are discussed elsewhere. Knowledge of basic organic chemistry is required throughout.

The book is clearly written and profusely illustrated. Occasional touches of humour help personalise the text. However, greater care in the relative emphasis given to specific examples would have improved the book. For example, several pages are devoted to the physical parameters of invertebrate oxygen-transporting proteins yet the discussion of ATCase in the section on allosteric enzymes is very short and has no illustrations. Other examples discussed, such as that of resilin, seem to contribute little to an introductory text.

Price also comments that, in writing an introductory text, he has resisted the temptation to delve deeply into certain exciting aspects of molecular biology. Nevertheless, I was disappointed that a number of the models discussed in detail seemed quite dated and were presented in an uncritical fashion, and also that such a recent book did not even allude to current topics such as recombinant DNA, DNA sequencing, the Ames test, or hybridomas. □

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Views of enzymes

Dennis Piszkiwicz

Steady-State Enzyme Kinetics. By Stanley Ainsworth. Pp.255. (University Park Press: Baltimore; Macmillan: London, 1977.) \$17.50. *Enzymatic Reaction Mechanisms.* By C. Walsh. Pp.978. (Freeman: San Francisco and Reading, 1979.) \$29.50. *Principles of Enzymatic Analysis.* Edited by H.U. Bergmeyer and K. Gawehn. Pp.260. (Verlag Chemie: Weinheim, 1978.) \$25.90.

In the past two decades our understanding of the nature and properties of enzymes has grown at a prodigious rate. The availability of texts describing these advances is now catching up with this growth. In recent years we have been treated to a large and somewhat redundant supply of texts on enzyme kinetics and mechanisms, albeit of varying scopes, lengths and quality. Three recent and different approaches to enzymology are examined here.

Kinetics observes catalysis in the time scale in which it occurs. It is the most important and popular tool available for the elucidation of enzymatic mechanisms.

Steady-State Enzyme Kinetics by Stanley Ainsworth presents an up to date survey of the mathematical gymnastics used to analyse enzyme kinetic data. It emphasises the methods of deriving models that describe the orders and methods of interaction of substrates and effectors to the catalytic molecules. The major fault of this text is that it really does not address the purpose of enzyme kinetics — the definition of the mechanism of catalysis — in as much detail as possible. It barely touches on subjects such as kinetic evidence of enzyme-substrate intermediates, pH dependencies, and so on, which can be related to the bond-forming and bond-breaking steps. It is unsettling to realise how so much sophisticated analysis can yield so little understanding of the forces being studied. In a review of Irwin H. Segal's *Enzyme Kinetics* (Wiley: New York, 1975), a text of similar scope, S.E. Halford (*Nature* 259, 255; 1976) noted that enzyme kinetics is a tool of biochemical research, not an intellectual discipline in its own right. Even as a tool, Segal's *Enzyme Kinetics* is more detailed and more accessible than this newer volume.

The results of the efforts of legions of enzymologists are brought together in *Enzymatic Reaction Mechanisms* by Christopher Walsh. This text, as the author notes in his preface, developed from his

Atmospheric Physics

J. V. IRIBARNE and H.-R. CHO

1980, xii + 208 pp. + indexes
Cloth Dfl. 40,- / US \$ 15.95
ISBN 90-277-1033-3

Atmospheric Physics is an elementary and comprehensive textbook on the terrestrial atmosphere and can be used for second and third year university courses. The book requires only the basic understanding of mathematics and such knowledge of physics as can be acquired in most first year general physics courses. It can be used in two ways. Firstly, a general review of atmospheric physics is provided for students who work or plan to work in other fields (such as geophysics, geography, environmental sciences, space research), but are interested in acquiring general information. Secondly, it will serve as a general and elementary introduction for students who will later specialise in some area of atmospheric science. Each chapter is concluded with a list of questions and problems which will help the reader to attain a more detailed insight into the various subjects discussed.

Microphysics of Clouds and Precipitation

HANS R. PRUPPACHER and
JAMES D. KLETT

1978, xvi + 714 pp.
Paper Dfl. 40,- / US \$ 19.95
ISBN 90-277-1106-2
Cloth Dfl. 85,- / US \$ 44.75
ISBN 90-277-0515-1

'This book no doubt will become a landmark in the realm of cloud physics, not only as an advanced textbook but also as a valuable reference. Its pages contain about as complete an exposition of current knowledge of the subject as one would hope to find anywhere.' Horace R. Byers, *Bulletin of the American Meteorological Society*

'The book, being a comprehensive, up-to-date description of the subject, is an excellent reference text for lecturers, students and researchers in atmospheric sciences, especially those interested in weather modification and air pollution.' Hans Mörth, *New Scientist*

'There is little doubt that this impressive book will be of central importance to cloud physicists and scientists working in related fields.' J. Latham, *Nature*

'I strongly recommend the book for scientific libraries, serious cloud physics students, and other scientists who need to go in-depth into this subdiscipline.' Dooyne Sartor, *Physics Today*

D. Reidel Publishing Company

P.O. Box 17, 3300 AA Dordrecht, Holland
Lincoln Building, 160 Old Derby Street,
Hingham, MA 02043, U.S.A.

own teaching, and it owes much to a course taught by his mentors R.H. Abels and W.P. Jencks.

The approach taken is to classify and present enzyme reaction mechanisms according to their chemical types: (1) group transfer reactions; (2) oxidation-reduction reactions; (3) elimination, isomerisation and rearrangements of substrate skeletons; and (4) the making and breaking of carbon-carbon bonds. This approach to organisation makes eminently good sense as it facilitates understanding of the basic patterns and principles underlying the reactions.

In presenting data which support the various enzymatic mechanisms, Walsh brings to bear evidence obtained from a wide variety of methodologies, including kinetic analyses, isotope exchange studies, protein chemistry, and the results of X-ray crystallography. The fundamentals of enzyme kinetics are presented, but they are treated superficially and are interspersed with discussions of various enzymes.

The grand scope of this text results in, at times, a superficial coverage of material pertaining to many mechanisms. It would have been preferable to have seen fewer examples of enzyme mechanisms examined in greater depth. Nevertheless, this original, ambitious work fills a need, and it is likely to be welcomed by both teachers and students of enzymology.

Principles of Enzymatic Analysis edited

by H.U. Bergmeyer and K. Gawehn is directed not toward the classroom but toward the laboratory. In this book enzymatic analysis means determination of the concentrations of substances with the aid of enzymes and determination of the catalytic activities of enzymes in biological materials. It is derived from a section of a larger work, *Methods of Enzymatic Analysis* by the same editor (Verlag Chemie and Academic Press, 1974), which has already gone through two editions. This revised version is a modest advance over its predecessor.

In this volume 20 authors have contributed to sections describing theoretical principles (including kinetics), the handling of biochemical reagents and samples, measuring techniques and instruments, and evaluation and assessment of experimental results. The derivation, content and format of this book will probably restrict its use to that of a reference work. As such the prospective user should be advised that it is limited in scope and often overly brief. This book is not intended for someone who wants to learn how enzymes function, but it may serve as a practical handbook for someone who is about to do his first enzyme assay. □

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Biochemistry for all occasions

Ernst Noltmann

Biochemistry: The Chemical Reactions of Living Cells. By David E. Metzler. Pp.1129. (Academic: New York, 1977.) \$22.50.

Modern Concepts in Biochemistry. By Robert C. Bohinski. Pp.600. (Allyn and Bacon: Boston, Massachusetts, 1979.) \$19.95

Biochemistry. By Frank B. Armstrong and Thomas P. Bennett. Pp.504. (Oxford University Press: New York, 1979.) \$19.95

Elementary Biochemistry, An Introduction to the Chemistry of Living Cells. By Julian Davies and Barbara Shaffer Littlewood. Pp.346. (Prentice-Hall: Englewood Cliffs, New Jersey, 1979.) \$20.75.

WITH biochemistry having become a serious (?) topic of prime time television talk shows, including guest appearances by dignitaries such as Linus Pauling, it is not surprising that this popularity is also reflected in an annually increasing onslaught of new textbooks of bio-

chemistry which present a confusing choice to both the instructor and the student. A most telling testimony is perhaps also the fact that this reviewer was asked to render an opinion simultaneously on four different textbooks. An attempt will be made to comment on the four books in the order indicated above which is according to decreasing size but which also reflects an order of value as perceived by this reviewer.

Metzler's coverage of the field is current and thorough. It is supported by many good illustrations and by superb bibliographies for each of the sections. The emphasis appears to be fairly even and the decision of the author to include many fascinating applications of biochemistry in special boxes set off from the main text, rather than to incorporate them into the narrative flow of fundamental material, is of great help to the student whose academic programme may demand a judicious use of study time. The detail of Metzler's text is both an advantage and a disadvantage. It makes reading slow for the novice. However, the writing is good and the style interesting. Moreover, the sentences are varied and well constructed — something rare for a science text. Also, on re-reading, the detail does not appear to be excessive and the opportunity to pursue

details through the extensive, up-to-date bibliography makes the book a good reference source. Metzler's book is definitely designed for the serious biochemistry undergraduate major or graduate student. This reviewer would place it as a text besides or even ahead of such greats as "White, Handler and Smith", "Mahler and Cordes" or "Lehninger."

Bohinski's book is, in general, written in a clear fashion. Individual chapters, although beginning at the level of understanding for non-majors, proceed with great detail which sometimes overwhelms the student, who may have difficulty placing the information in the proper perspective. In our department, the text serves a one-quarter course for non-biochemistry science majors for which it was found to be a good background text because of its emphasis on structure-function relationships and on regulation as a key to understanding metabolism. It may be suitable also for a two-quarter course if students come with a solid background in biology and chemistry. Some definitions appear to be misleading. For example, this reviewer believes the explanation of quaternary structure (p.145) to be incorrect. The desire for completeness has led the author on occasion to use metabolic schemes too complicated to be didactically useful (for example, fatty acid synthesis). Some students feel that additional examples of problem-solving would be helpful for the understanding of pK_a 's, ΔE° , and the intricacies of peptide sequencing. Of all the four texts the print of Bohinski's book is the most difficult to read, especially when it appears in the italic mode. As a special point, this reviewer enjoyed the essay by Morowitz on the "Six Million Dollar Man" included in the Preface. It serves well to put it all in perspective.

The book by Armstrong and Bennett presents biochemistry at a level and with a degree of detail adequate for a service course for non-majors for whom biochemistry is more an ancillary requirement than a discipline of interest for its own sake. The coverage of the various biochemical topics varies and seems to be influenced by the preference of the authors. The chapter on biogeochemical cycles is unusual. The treatment of enzymes and nucleic acids is thorough. Repair and recombination of DNA are dealt with in a very up-to-date fashion. A separate chapter on nutrition is particularly valuable. In fact, it is most deplorable that this vital area of biochemistry is neglected by most textbook authors. Unfortunately, practically no mention at all is made of fatty acid synthesis, and synthesis, in general, is given little attention. Frequent reference is made to medical relevance which is usually of interest to students, including those who are not premeds. This reviewer liked the historical introduction as well as the

summaries after each chapter, the latter allowing students to place the various subfields of biochemistry in relation to each other.

Finally, *Elementary Biochemistry* by Davies and Littlewood is quite different from the other three books reviewed here. While the authors warn that the title may be misleading, this reviewer feels that the simplistic treatment may be just that, namely misleading. A treatment of enzyme kinetics without ever using the word "kinetics" or without mentioning Michaelis and Menten or Lineweaver and Burk appears to be less than "elementary". However, if perused only by those whose only aim is to get a "feeling" of biochemistry "for fun", it is a nice means to seduce perhaps the non-science

student to probe more deeply into what biochemistry really is. The descriptions of some biochemical techniques are very good and may induce those whose interests are aroused to look further. Interestingly, the problem sets provided by the author appear to be much more difficult than the text suggests. Students will need considerable help from the instructor and from teaching assistants in order to be able to solve them. Nevertheless, the book has a good format. It reads easily, the summaries are clear and, as a whole, the book may help newcomers to sense the excitement of "being in biochemistry". □

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Basic chemistry

James Viers

Fundamentals of Chemistry. By Fred H. Redmore. Pp. 711. (Prentice-Hall: Englewood Cliffs, New Jersey, 1979.) \$18.95. *A Basic Math Approach to Concepts of Chemistry.* By Leo Michaels. Pp.312. (Brooks Cole: Monterey, 1979.) *Fundamentals of Chemistry.* By E. Kostiner and J. R. Pea. Pp.472. (Harcourt Brace Jovanovich: New York, 1979.)

IN a foreword to *Fundamentals of Chemistry*, by Fred H. Redmore, the statement is made that the text was developed out of an attempt to expand and upgrade *Basic Chemistry* by Seese and Daub into a general chemistry text. I do not feel that either the expansion or the upgrading is sufficient to place *Fundamentals of Chemistry* into the category of a desirable general chemistry text which will properly prepare students for higher level chemistry. While most of the topics normally found in general chemistry texts are included here, the treatment of a number of topics is somewhat cursory. The kinetics chapter, for example, totals 11 pages (as does the chapter on co-ordination compounds) including summary, exercises and problems and a page of objectives. Perhaps the biggest shortcoming in the book is the number and type of end-of-chapter problems. The chapter on kinetics mentioned previously has only two exercises (of a qualitative nature) and three rather trivial problems. Other chapters do contain more problems many of which have multiple parts. However, almost all of the problems are basically of the drill and practise type, with few challenging ones and very little application of chemical principles to relevant or practical types of problems. An instructor who uses this text will very likely be forced to use

supplementary problems extensively.

There are a large number of general chemistry texts on the market today which are superior to *Fundamentals of Chemistry* for use in the main line course which is structured to prepare science and engineering students for organic and/or physical chemistry. If this text has a market, it might be in the introductory chemistry course currently offered as a year-long terminal course at many schools. The book is well written and very attractively designed in a two-colour black and red format. It can be recommended for those courses which are intended to be terminal and for a course in which the instructor wishes to present chemistry in a rather qualitative fashion.

A Basic Math Approach to Concepts of Chemistry, by Leo Michaels, is a workbook, basically of the programmed type, in which each unit is broken into parts and frames. Questions within each frame are designed to enable the student to master each idea before moving on to the next frame. The purpose of the text is purportedly to teach students who are taking or preparing to take a college course in chemistry the mathematical skills necessary for such a course. Some of the mathematical topics covered, however, border on the absurd for any student in college. Unit 1, for example, deals with the naming of whole numbers (example: Write the number that goes with the word name eight thousand). Unit 2 treats naming, adding, subtracting, multiplying, and dividing of decimal numbers, while unit 3 covers the definition, adding, and subtracting of signed numbers. Unit 4 teaches the student to write numbers in power-of-ten form and to multiply and divide numbers in power-of-ten form. It is inconceivable that any college student who has graduated from high school or even elementary school should need to review mathematical material of this nature. The remainder of the 14 units are devoted to topics which are somewhat more advanced and more directly related to calculations of

a chemical nature. These topics, however, are covered at a rather superficial level. The only type of student who is likely to benefit from this workbook is a student whose high school mathematical training is virtually nonexistent. If the student really needs exposure to many of these topics, it is very doubtful that he will be able to compete successfully in a true college level general chemistry course.

Fundamentals of Chemistry by E. Kostiner and J. R. Rea, is designed for a one term, preprofessional introductory chemistry in a straightforward, easily readable style. The book is attractively designed in a two-colour black and red

format which makes it very easy to read. Major topics found in most full year texts which are not presented here include: kinetics, thermochemistry, nuclear chemistry, co-ordination compounds and a discussion of the transition elements. A reasonably large number of worked-out example problems are included in most of the chapters as well as a fair selection of end-of-chapter problems. Fairly extensive chapters on organic chemistry and biochemistry (about 13% of the text material covers organic and biochemistry) are attractive features of the book and should appeal to the audience for which the text is designed. Historical and relevant

sidelights are prominently displayed throughout the text and also add appeal. The book emphasises the basic concepts of chemistry, however, and is not of the 'cutesy' variety with cartoon-type drawings which has recently become popular for use in the abbreviated one term general chemistry course. This text is highly recommended for instructors who wish to present a one term course in general chemistry which emphasises the basics of chemistry. □

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Experimental organic chemistry

P.F. Schatz

Experimental Organic Chemistry. By David Todd. Pp.346. (Prentice-Hall: Englewood Cliffs, New Jersey, 1979.) \$13.95. *Techniques and Experiments for Organic Chemistry*. Third edition. By Addison Ault. Pp.442. (Allyn and Bacon: Boston, 1979.) \$16.95.

RECENTLY, there has been a flood of organic chemistry laboratory manuals from the book publishers. Among these books are revised editions of previous manuals and a few brand new manuals. On perusing the manuals it is sometimes difficult to avoid a sense of *déjà vu*, for there is a great deal of overlap in the experiments detailed. One new book that does not suffer in this way is *Experimental Organic Chemistry* by David Todd. Although there are a few familiar faces, this collection of fifty-eight experiments contains many new experiments for the undergraduate. With this large number to choose from, an instructor should have no difficulty in selecting enough interesting and instructive experiments to fill an introductory organic laboratory course. Examples of common organic preparations, Grignard reaction, Friedel-Crafts acylation and Fischer esterification to mention a few, are included, as well as some not so ordinary preparations such as the Peckmann coumarin synthesis and the Rothmund reaction for the preparation of tetraphenylporphyrin.

Unlike many laboratory manuals, which present laboratory techniques separate from the experiments, Todd introduces the techniques as an integral part of a specific experiment. For example, column chromatography is explained and

demonstrated in an experiment on extracting pigments from spinach. As a consequence of this approach, one could be locked into doing a certain core of experiments in order to introduce the basic techniques. The experiments are detailed enough to be carried out by the inexperienced undergraduate student. The discussions of the techniques are concise and give enough information for the student to understand the how and the why of the techniques. The section on NMR and IR spectroscopy is very short (7 pages) and just covers the basics. This would not be the book for a laboratory course that is heavy on instrumentation.

Among the good points of the manual are the detailed instructions conveyed in a very conversational style. This, coupled with a section entitled "Most Common Errors", which appears after each set of instructions, should lead to good results for most students. Another favourable point is that many of the experiments and compounds encountered in the experiments can be related to familiar, naturally occurring materials. The NMR and IR spectra of thirty-five compounds are illustrated, but they appear to be retracings of spectra as opposed to reproductions of actual spectra (the coupling in the methyl triplet of the ethanol spectrum on page 97 does not appear to be symmetric as it should be). Also, the solvents used to prepare the NMR samples are not noted on the spectra.

There is some inconsistency in the warnings regarding hazardous properties of the chemicals. For example, the toxicity of benzene is noted in Experiments 25 and 33, but no mention of it is made in an earlier one — Experiment 13, the Grignard synthesis of monodeuterated benzene. Also in Experiment 13, 1,2-dibromoethane is suggested as a reagent for starting reluctant Grignard reactions and no warning is given regarding its toxicity. This compound is on the OSHA tentative Category 1 carcinogen list, as is benzene. Thus, any instructor using this book, or any other laboratory manual for that

matter, should check to see if the hazard warnings included need to be supplemented or updated.

Among the revised editions of organic chemistry manuals is *Techniques and Experiments for Organic Chemistry* by Addison Ault. This is an updated and expanded version of a good laboratory manual. Better than one-half of the book is devoted to describing techniques and apparatus for the organic chemistry laboratory. This is done thoroughly and clearly and is illustrated with large, accurate drawings. Modern spectroscopic techniques, NMR, IR, UV and mass spectrometry, are dealt with extensively.

The second half of the book is a compendium of experiments demonstrating the techniques and the characteristic reactions of organic compounds. The experiments are divided into four groups: separations, transformations (one-step procedures), synthetic sequences and projects. The separations, transformations and synthetic sequences are all well worked out, carefully detailed experiments which provide good working experience with organic chemistry.

For the more adventurous and skilled, there are the projects. These are procedures excerpted directly from the primary sources in the chemical literature. The compounds included are natural products, are theoretically interesting, or have unusual physical properties. The ability to reproduce procedures directly from the chemical literature is very important to the organic chemist.

In summary, Ault's book is a well written modern, informative manual that should be looked at seriously by anybody who is considering using laboratory manuals. □

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Basic botany

Ralph S. Quatrano

Botany: A Brief Introduction to Plant Biology. By Thomas L. Rost, Michael G. Barbour, Robert M. Thornton, T. Elliot Weier and C. Ralph Stocking. Pp.344. (Wiley: New York and Chichester, UK, 1979.) \$20.15. *Plants: Basic Concepts in Botany.* By Watson M. Laetsch. Pp.510. (Little, Brown & Co.: Boston, Massachusetts, 1979.) \$17.95. *Instructor's Manual to Accompany Plants: Basic Concepts in Botany.* By Watson M. Laetsch. Prepared by Mary S. Manteuffel. Pp.121. (Little, Brown & Co.: Boston, Massachusetts, 1979.) \$17.95. *Instructor's Biology.* By Kingsley R. Stern. Pp.482. (Brown: Dubuque, Iowa, 1979.) \$13.95. *Laboratory Exercises/Biology of Plants.* Fourth edition. By H.L. Dean. Pp.326. (Brown: Dubuque, Iowa, 1978.) \$9.95. *The Green World, An Introduction to Plants and People.* By Richard Klein. Pp.512. (Harper and Row: New York, 1978.) \$14.95.

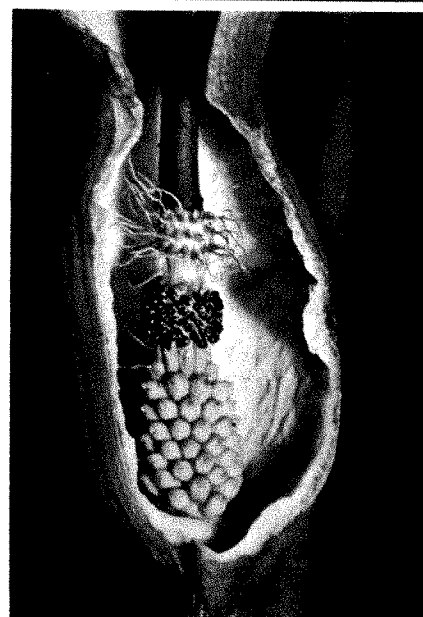
MOST recent textbooks of plant science that are aimed at beginning undergraduates can be grouped into two classes: those that approach the subject from a more traditional and classical manner, and those that popularise plants by emphasising their uses and values to society, that is, ethnobotany. The former tend to be used in courses for agriculture, forestry and biological science majors. Such courses usually serve as a technical framework upon which is based an advanced curriculum in morphology, physiology, ecology and systematics. The other texts began to appear during the last decade and tend to be used in non-major courses and in two-year colleges where exposure to plants, their diversity and value to society are stressed. The texts reviewed here represent a wide choice between these categories.

One of the best and more popular classical texts for majors by Weier *et al.*, *Botany*, has now been published in a shortened version, *Botany: A Brief Introduction to Plant Biology* by Rost *et al.* In many ways this text is a revision of the larger fifth edition (1974), since much of the subject matter has been rewritten and updated and new drawings and figures added. It is still a basic plant science text with no major change toward a more simplified or popularised treatment of the science. (Generally, the selection of topics to retain and condense was good, considering the difficult job of reducing a 642-page text to 344 pages (a slightly larger format, however).) The bacteria and virus chapters were dropped and extensive coverage of certain topics was reduced (for example, CAM metabolism and C_4 photo-

synthesis). However, all the basic botanical subjects are well represented in breadth and depth in the revision. The illustrations are excellent, especially the drawings. The basic organisation and groupings of topics are flexible; subject matter usually covered together can be assigned as units (chapters/appendices) rather than scattered pages. The one minor exception is the coverage of genetics where the molecular aspects of gene expression are covered within the chapter on metabolism separate from mendelian genetics. This is a well written text, with a good glossary, chapter summaries and a basic chemistry appendix. This text couples a sound treatment of the essential topics to be presented in an introductory plant science course with conciseness and brevity. It would be a good text in a short course for majors, the "plant" text in an Introductory Biology series and an alternative to a popularised treatment of plant biology for an introductory course at a junior or community college.

Laetsch has written a text for majors (*Plant: Basic Concepts in Botany*) that presents the traditional topics but in addition includes a considerable emphasis on the role, uses and importance of plants in the lives of students. His approach is to present topics in a context with which students are familiar and to stress the importance of plants in their lives. Energy is introduced in the first chapter within the broad framework of the biosphere which includes mention of food chains and energy transfer within ecosystems by use of common examples. This chapter and the following one entitled "Agricultural Revolutions" will impress on the reader the importance and usefulness of plants to their existence and the future of mankind. A fairly typical coverage of the plant kingdom follows in the second part of the text which exposes students to the diversity of plants. Taxonomic principles are covered using Angiosperms that everyone knows, that is, "the supermarket plants". This is extended in the Appendix (Supermarket Taxonomy) in which a brief taxonomic description is given for 16 selected families of popular dicots and monocots. The last chapter of this section deals with "People's Plants", in which 16 plants are presented in a historic and socio-economic framework to acquaint students with those "plants that keep our culture running" (wheat, sugar cane, rubber, coffee, tobacco).

These first two sections should serve to heighten the interest of the students to delve into the last two sections dealing with the structure/function and evolution of seed plants. Here the organisation of the material is similar to most other texts and will allow an instructor considerable flexibility in covering the basic and more popular topics in any sequence he desires and still be able to assign individual chapters. However, the depth in which most topics are covered (for example, photosynthesis/respiration and survey of



The chamber or spathe of the cuckoo pint traps flies carrying pollen from other plants, so aiding its fertilisation.

the plant kingdom) is not as extensive as in other texts for majors such as (*Biology of Plants* by Raven, Evert and Curtis). Little chemistry is included (no structural formulae) and hence the freshman with no chemistry background would have few problems with this text. The author has introduced basic botanical principles in a way that will stimulate the student's interest and excitement in the plant sciences. The text is well written and includes extensive references, chapter summaries and review questions, a glossary, as well as 1-2-page supplements scattered throughout the book. These add more depth and help explain abstract concepts that might interfere with the flow of the text. A helpful *Instructors Manual* is included. The text is different from others now on the market but in a way that does not eliminate its use in any traditional plant science course or in a non-major course. I suspect this text can be adopted by a wide range of existing courses at different types of educational institutions.

The text *Introductory Plant Biology* was written by Kingsley R. Stein to "emphasize current interests (of students) without giving short shrift to botanical principles". Stern's approach is that the basic science of botany is more meaningful to beginning students if its relationship to the more applied fields (for example, horticulture) is emphasised. This text, like the one by Laetsch, falls between the two extremes of introductory plant texts. For the most part, the integration of applied botany into the main part of the text does not constitute much more of an emphasis than most other traditional texts. However, 90 pages of Appendices represents most of the emphasis on practical botany. This includes a section on the use of biological controls as an alternative to chemical control by pesticides and herbicides, a

From *Color in Plants and Flowers* (Everest House: New York, 1978)

listing and brief description of useful plants (for example, wild edible plants, poisonous and medicinal plants), home gardening tips and information about conditions for optimal growth of common houseplants. Also included in the Appendices, however, is a section with more details of DNA structure and function, photosynthesis and respiration for those who wish a more in-depth treatment. The first 10 chapters of the text represent the areas of morphology, physiology, development and genetics while the remaining 11 chapters deal with classification, ecology and a survey of the plant kingdom including bacteria, blue-green algae and viruses. One of these chapters, entitled "Flowering Plants and Civilization", discusses 15 well known families and, using representative examples of each, Stern briefly describes their ties with various cultures as well as their economic importance.

Review and discussion questions follow each chapter as well as a glossary at the end of the text. A well illustrated and proven (fourth edition) lab manual is available from the same publishers (*Laboratory Exercises. Biology of Plants* by H.L. Dean). Although this book can be used in major courses, the depth and level of coverage of topics plus the nature of the Appendices suggest a probable wider acceptance in the non-major or non-science courses.

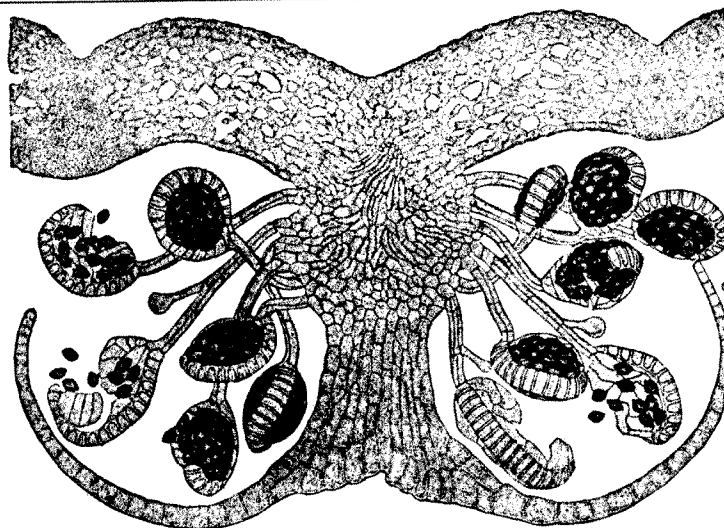
The *Green World. An Introduction to Plants and People* by Richard Klein is clearly different from the above texts. It is a unique contribution to botanical books and represents the opposite end of the spectrum from traditional, technical texts. His approach is clearly a historical one in presenting plants as an important component of various cultures. The author's view point is that "through an examination of the green world we can better understand the modern world and

the civilization from which western culture has evolved". With this objective his text is quite successful. He presents fascinating and exciting historical and cultural facts about plants that will no doubt interest any reader. Chapter titles include Economics and Politics of Food, Plants in Religion, Medicinal Plants, and subheadings such as Spices and Savory Herbs; Plants of Superstition, Myth and Ritual; Herbs, Herbals and Herbalists; and Botany of Coffee. It is not a popularised or strictly applied text but more of a scholarly, intellectual presentation of the role plants have played and will continue to play in society. It is flavoured with the personality of the author which is quite refreshing. However, the coverage of the topics normally found in the classical texts is brief and simplified and mainly confined to scattered one- or two-page essays or supplements to the major chapters. Conspicuously absent are coherent sections on morphology, mendelian and molecular genetics, survey

of the plant kingdom and respiration to name a few. With the exception of a few references at the end of chapters, there are none of the usual aids such as chapter summaries or review questions and a glossary. I doubt if this book will be used as the sole text in a beginning botany course for science majors. It would have merit as supplemental reading in such a course, in a non-major course, or as a text in a seminar course for advanced botany majors interested in a different and valuable perspective.

Instructors of plant science have quite a diversity of good new texts from which to choose the vehicle to convey the science and excitement of botany to young, eager minds. □

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Vertical section through the sorus of the fern, *Dryopteris*, showing sporangia in various stages of development.

From *Intermediate Botany* (Macmillan: London)

Modern virology

General Virology. Third edition. By S.E. Luria, James E. Darnell Jr, David Baltimore and Allen Campbell. Pp.578. (Wiley: New York and Chichester, UK.) \$23.50.

THIS excellent textbook uniquely fills a major need in the biological sciences: a single volume to introduce the many facets of modern virology. It is clearly written and well organised, so that it can be used essentially as the sole reference source for a general virology course.

As expected from the credentials of the authors, the book heavily emphasises the

P.C. Kimball

molecular level of knowledge about viruses. To take full advantage of this text, students should have had some previous exposure to the basics of molecular biology from a biochemistry or genetics course (or both). Certain chapters summarise the principal features of gene expression and animal cell biology; however, as indicated by the authors, these helpful reviews cannot substitute for a more thorough treatment of these topics prior to using the text. Some previous exposure to advanced mathematics, especially an introduction to statistical concepts, would also be useful to the students. Thus, this text is recommended primarily for advanced

undergraduates or graduate students majoring in the biological sciences. To read the entire text in one academic quarter will be an intense experience for such students; those in the semester system will still find the breadth and depth of material challenging. For these reasons this text is not recommended for an introductory course for less advanced undergraduates or those not majoring in a molecularly orientated programme; unfortunately, no text suitable for these latter students is presently available to provide the same broad coverage of the field on a more introductory level.

The book is well documented with an 80-page bibliography. Therefore, it will continue to serve the students as a detailed reference book. Although important new developments are frequent in virology in

this age, the text is thoughtfully written to prevent rapid obsolescence. For example, the book went to press just before the first convincing demonstrations that RNA splicing is required for production of mRNA in several mammalian systems. Nevertheless, the background for this finding is carefully developed in discussions on the relationship between cellular hnRNA and mRNA, and in the summaries of known details of viral mRNA production. In fact, the authors correctly predicted that the adenoviruses would be one of the most productive systems for clarifying precursor-product relationships in mammalian mRNA

synthesis. In general, where significant controversy exists, the authors present a balanced view and try to indicate the experimental approaches most likely to lead to resolution of the issue. Another strong point is the thorough description of most virological methodology in frequent use today.

The one major deficiency of this text for some purposes may be the relative lack of emphasis on the overall effects of viruses on the host, especially in higher organisms. Thus, the nature of viral diseases and defence mechanisms in animals are given only summary treatment, while there are only hints of the importance of plant and

insect pathogens for agriculture. Many students continuing their education in the biological sciences may encounter some of these topics in other specialised courses, depending on their interests; but some teachers may wish to supplement the text in these areas for the sake of providing greater appreciation of the more biological aspects of virology, to complement an otherwise complete introduction to the field. □

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Basic biology of malignancy

Joseph R. Bertino

Fundamentals of Oncology. By Henry C. Pitot. Pp.192. (Marcel Dekker: New York, 1978.) \$9.95.

THIS concise textbook, which stems from a course given at the McArdle Institute for Cancer Research at the University of Wisconsin, is directed toward non-physician scientists, undergraduate and graduate students, and postdoctoral fellows in the biomedical sciences. Actually, medical students would also find in the book a great amount of material not covered adequately in their courses in pathology and medicine.

Since the book is written by one author, the writing is consistent throughout, and the 12 chapters are all equally well done. The author writes in a lucid direct style, and is able to present the reader with the important aspects of each topic with sufficient definition and background so as to be easily digested. Each chapter is referenced; the student who wishes to read in more depth on a particular topic will find all of the key references. The titles of articles are included, so that although the text is not documented (which would diminish its readability) the appropriate references for each section can be found. The author is an experimental pathologist and biochemist, and I found the chapters on aetiology and pathogenesis of cancer, and the biochemistry of cancer especially well done. Since these chapters comprise most of the message, the book is very successful. Other textbooks may contain

more information on specific topics, but none present so much information in such a well written, concise fashion.

The book does not cover topics such as cancer diagnosis or cancer treatment (chemotherapy, surgery, immunotherapy and radiation therapy), but the student who masters the material in this book will have the background to understand these more clinical topics.

In summary, this book is highly recommended for both undergraduate or graduate students as well as physicians who wish to know more about the basic biology of malignancy. □

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History of biology

Dov Ospovat

A History of the Life Sciences. By Lois N. Magnier. Pp.489. (Marcel Dekker: New York, 1979.) \$23.50.

LOIS MAGNIER'S *History of the Life Sciences* was written as a textbook for undergraduate courses in history of biology (it does not pretend to encompass the history of medicine). The discussion effectively begins with the pre-Socratic philosophers and continues into the "post Watson-Crick period" in the study of genetics. Some 70 pages are devoted to ancient and mediaeval science and another 40 pages to the Renaissance and Scientific Revolution. Most of the remaining three-quarters of the book treats the development of embryology, cell theory,

microbiology, physiology, and evolution from the seventeenth century to around 1900. Only in the last two chapters, both of which are concerned with genetics, is there any sustained discussion of twentieth-century biology. Scientific societies and microscopes are each accorded a brief chapter. There are some curious omissions. For instance, little attempt is made to relate seventeenth and eighteenth century biology to the Scientific Revolution. In the chapter on physiology Helmholtz is only mentioned, while Ludwig and Du Bois Reymond are completely ignored.

Magnier's basic technique is as follows: under each chronological or subject heading, she discusses the individual scientists deemed to have contributed to the advance of biology. One result is that many key issues — such as the philosophical and religious concerns of seventeenth, eighteenth, and nineteenth century biologists, and the arguments amongst nineteenth century naturalists and physiologists over appropriate styles of

explanation — are inadequately treated, in a piecemeal fashion in the biographical sub-sections.

The biographical approach is no doubt convenient for author and students. Likewise, because it conforms to the pre-conceived notions of most undergraduates, presenting science as progressing toward the present is perhaps the easiest way to teach the history of science. But since it is now pretty generally agreed that this is a wholly unsatisfactory way of understanding the past, it is strange to see it adopted as the organising principle of a new textbook.

Magnier often seems to be interested chiefly in whether a scientist was 'progressive' or 'retrogressive', as defined by mid-twentieth century knowledge and attitudes. Thus we are told that "although preformation theory impeded progress in embryology, it had one virtue. Preformationists generally rejected spontaneous generation" (page 188); that La Mettrie "paved the way for more

modern biological theories" (page 307); and that the *Origin of Species* was "a clear statement of the fact and mechanism of evolution and was quite convincing to readers with open minds" (page 385). Rather than attempting to present Haller's thought in its eighteenth century context, Magner praises him for his "restrained and modern spirit" (page 305).

The most serious weakness of the book is that Magner has made very little use of recent scholarship. As a sub-field of the history of science, the history of biology has come into its own in the last 20 years, yet Magner relies largely on much older works, predominantly books. Consequently, the presentation is often seriously deficient, given the current state of historical knowledge. This is especially true of the chapters on microbiology and biogenesis, evolution, and embryology.

I found numerous errors, some minor, others serious: the Reign of Terror is said to have occurred in 1789 (page 361); the relationship of von Baer's laws to the theory of recapitulation is mis-stated (page 204); the word 'biology' is said to have been coined as a replacement for 'natural philosophy' (page 405); and it is claimed that "taken as a whole" Buffon's writings "contain all the elements of the Darwinian synthesis" (page 358). □

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Historical and physical geology

Hermann W. Pfefferkorn

Earth History and Plate Tectonics: An Introduction to Historical Geology. Second edition. By Carl K. Seyfert and Leslie A. Sirkin. Pp.589. (Harper and Row: New York, 1979.) \$18.95. *Geological Evolution of North America.* Third edition. By Colin W. Stearn, Robert L. Carroll and Thomas Clark. Pp.556. (Wiley: New York and Chichester, UK, 1979.) \$25.90. *Interpreting the Earth.* By Robert R. Compton. Pp.554. (Harcourt, Brace Jovanovich: New York, 1977.) \$16.95. *Physical Geology.* By L. Don Leet, Sheldon Judson and Marvin Kauffman. Pp.490. (Prentice-Hall: Englewood Cliffs, New Jersey, 1978.) \$24.25. *Physical Geology.* By Bob F. Mallory and David N. Cargo. Pp.538. (McGraw-Hill: New York, 1979.) \$16.95.

OUR understanding of the history of the Earth has changed dramatically with the appearance of plate tectonics as the unifying theory of geology. This change had to influence the teaching of historical geology profoundly. In pre-plate tectonic times historical geology was taught as a survey of local lithostratigraphies with endless lists of stratigraphic names, as a survey of the evolution of life, or ideally as a combination of both. The large number of lithostratigraphic names often made historical geology an exercise in memory. With the change in emphasis from acquisition of encyclopaedic knowledge to understanding geological concepts, many geology departments dropped undergraduate courses in historical geology. However, plate tectonics has given us a theoretical framework for the physical aspects of historical geology and simplified explanations for many geological phenomena. Thus, historical geology can now be taught with an emphasis on principle rather than detail. The first two books are examples of this new approach.

Seyfert and Sirkin present principles in the first seven chapters in 148 pages (evolution, geologic time, ancient environments, correlation and facies, plate tectonics). One chapter is devoted to cosmology and origin of the Earth. Chapters 8 to 14 (387 pages) deal with historical geology proper. Each of these chapters treats a large time interval like the Precambrian or late Palaeozoic. Only the last two chapters, on the Tertiary and Quaternary, are devoted to one period each. The Phanerozoic time scale and an extremely brief description of animal and plant groups are presented in Appendices A and B. Seyfert and Sirkin emphasise the history of North America but also treat the

rest of the world. Text figures are numerous and well selected. The maps of continent positions at different times are shown in an ingenious projection. Many maps and cross sections were apparently created or modified for this book and are easy to understand. The history of life is well integrated with the other aspects of historical geology.

Stearn, Carroll and Clark begin their book with a discussion of plate tectonics in Chapter 1 followed by seven chapters (147 pages) on principles (orogenesis, stratigraphy, correlation, time, cosmology, palaeomagnetism, evolution). The following chapters (377 pages) are grouped into four parts; the craton, the Cordillera, the Appalachians, and the Arctic and Cenozoic Ice Age. The discussion is restricted to North America and each part of the continent is taken as an example of one geotectonic province. For each area the geological history for the most significant time interval is given. Thus, some geological periods are treated more than once. An appendix gives concise descriptions of plant and animal groups. The book contains many excellent figures. I find it refreshing to see numerous figures from Canadian sources, which are not often used to illustrate textbooks. Graphs are well executed and two-colour printing improves clarity.

Both texts have about the same number of pages, use the same space for introductory chapters and main body, and are equally well suited to teach historical geology. Both make fascinating reading. The difference lies in their approach: time sequence with world-wide coverage (Seyfert and Sirkin) versus a case history of North American geological provinces (Stearn, Carroll and Clark). On the undergraduate level, historical geology has usually been taught as the second geology course after physical geology. The books discussed are written for just that sequence. However, while plate tectonics has unified many seemingly disparate aspects of historical geology, moving plates in ever changing configurations are a formidable matter for undergraduates to master so early in their geological education. Therefore, it might be more appropriate in the future to teach historical geology as a higher level course in the senior year. Both books could be used for such a course with some supplemental reading.

The other three books are all intended as an introduction to geology. They achieve this in different ways. Compton starts to analyse a certain area and to discuss the geological principles active there. The other two books follow a more classical approach. Leet, Judson and Kauffman begin with internal processes of the Earth and Mallory and Cargo with external ones. The surface processes are, of course, more familiar to the student and Mallory and Cargo have developed the most coherent sequence of chapters of the three books.

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Their book is further written in a language which is easy to read. All the figures are easy to understand and many of them are original. In summary, the text by Mallory and Cargo can be used well in a course with many non-majors. Leet, Judson and Kauffman's book is written for science majors and geology majors. It is well written, rather complete in its coverage and gives extremely instructive examples and case histories. The photographs of outcrops and landscapes are impressive. Graphs are easy to understand and well done. Compton integrates principles with the discussion of specific areas and examples of scientific studies. He begins with surface processes and situations which

have not only geological but also environmental significance. This is clearly the most original of the three textbooks discussed here. A course based on this book might motivate students of geology who might be turned off by other textbooks. The colour photographs of outcrops, rocks and minerals would be especially helpful in a course without a laboratory part. Nevertheless, the data presented are detailed enough for the highest level introductory course.

In each of these three books I found some graphs and explanations similar to the ones I use in teaching. Selecting one of the three as a textbook would be a difficult choice. The decision would partly be based

on the kind of students who would predominate in the class. The other consideration would be how much one would be willing to adapt the course to the textbook. Compton's book would be best suited if course and textbook would coincide in the sequence of their treatment of topics. Leet, Judson and Kauffman's book could most easily be used in a course with a totally different sequence of lectures. Mallory and Cargo's book stands between the two others in this respect. □

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Igneous petrology

S. A. Morse

The Interpretation of Igneous Rocks. By K. G. Cox, J. D. Bell and R. J. Pankhurst. Pp.450. (George Allen and Unwin: London, 1979.) Hardback £18; paperback £8.95.

AFTER half a century of near-starvation, students of igneous petrology are being presented with a succession of books that pay serious attention to phase diagrams. This book furnishes a square meal, devoting to phase diagrams more than thirty per cent of the text. And, joy of joys, at last we have a decent treatment of trace elements and isotopes, which occupy fifteen per cent of the text. The remaining half, liberally sprinkled with boldface where definitions occur, is taken up with such matters as intelligent classification, variation diagrams, fractionation, a respectable amount of petrography, experimental and controversial studies of natural rocks, volcanology, and plutonology (complete with flow equations). Appendices include directions for norm calculations and for plotting rock analyses in O'Hara diagrams. Exercises after each of the fifteen chapters are furnished with answers at the end of the book.

These blessings are not unmixed. Much space devoted to hypothetical phase diagrams could more usefully have been allotted to additional real ones with equivalent lessons as to their use. The quaternary projections may be overdone. The geometry of fractional melting is given short shrift, and its quantitative possibilities are mistakenly denied. Many readers will groan to find an unhappy treatment of variance which yields univariant points and divariant lines in 1-atm phase diagrams. Contrary to the

implications in the text, the isobaric restriction does rigorously reduce the variance by one, and this pitfall of nomenclature could have been avoided by use of an appropriate notation, such as F_p , to denote the restriction. Most of the classical phase diagrams are taken directly from the ancient literature without revision: we find albite melting variously at 1,120°C and 1,100°C rather than 1,118°C as determined by Greig and Barth in 1938. Kushiro's revision of Di-An-Ab is ignored except for being mysteriously ascribed to An-Ab. The incongruent melting of fayalite is not mentioned, nor is there any discussion of the role of oxygen in igneous fractionation. Carnegite is said to "exsolve" on a reaction loop.

But one should not look a gift horse in the mouth. The text focuses on methodology and is offered to petrologists

of all ages. As an unintentional test of its utility, the review copy was thoroughly consulted, marked up and leaned upon during a recent seven-hour research conference, and it gave good service. The treatment of trace elements in the mantle and in magmas, and of radiogenic and stable isotopes, is a masterpiece of concise, clear erudition. Mantle isochrons and hydrothermal Taylor engines are not neglected. The text is plainly written throughout and easily accessible to students. In sum, this book is a most refreshingly welcome and, indeed, indispensable addition to the still pitifully small genre. □

S. A. Morse is Professor of Geology at the Department of Geology and Geography, University of Massachusetts, Amherst, Massachusetts.

Chestnuts of the '40s

Brian J. Skinner

Exploration and Mining Geology. By William C. Peters. Pp.696. (Wiley: New York, 1978.) \$26.50. *Economic Mineral Deposits.* Third edition. By Mead L. Jensen and Alan M. Bateman. Pp.593. (Wiley: New York, 1979.) \$28.75.

THE generation of mining and exploration geologists trained during the 1950s had the delightful experience of reading H.E. McKinstry's eloquent volume *Mining Geology* (Prentice-Hall: Englewood Cliffs, New Jersey, 1948). There were no successors to inspire students of the '60s and '70s, but it is probable that William Peters' new book *Exploration and Mining Geology* will fill the role for the generation of the '80s. The need for a successor to

McKinstry's volume has become acute; the profession has grown enormously as mineral production has increased; mining methods have become more complex; many new techniques of geochemistry and geophysics have been added to the modern prospector's arsenal; the sophistication of geological information has grown dramatically and the economic conditions of today are far removed from those of the 1940s when McKinstry wrote his book.

Peters does not share McKinstry's mastery of English and his wry wit but he has prepared an admirable volume that the advanced student will find both pleasant and informative to read and that will also serve as a reference for the practising geologist. Throughout, he emphasises the observational and practical while at the same time urging the reader to keep an open mind. With good reason; new ways of looking at old ideas have found a lot of ore. The heart of the book, and the place where Peters' extensive experience shines through, is a nine-chapter section discussing what mining and exploration

geologists do and how they go about their business. But there are also concise and authoritative discussions of the origins and distribution of mineral deposits, of the engineering and economic aspects of mining plus descriptions of the way exploration programmes are organised and prospects evaluated. The volume is a mine of information and a pleasure to read.

How appropriate it would be to measure equal enthusiasm for the latest edition of another chestnut of the 1940s — Bateman's *Economic Mineral Deposits* — the book so many of us studied before we read McKinstry. When Bateman died in 1971, he had already started revising the volume. Perhaps he had delayed so long because the task of a total revision was beyond him; possibly he realised that the format of the volume, which dated back to the teaching pattern of his mentor, J.D. Irving, had become too cumbersome. Whatever the reason, when he did realise the task was beyond him, he asked his friend and protégé M.L. Jensen, formerly at Yale, now at the University of Utah, to complete the task. It was, in reality, a request to fit an old but successful format to a very different and rapidly growing field.

The experiment has not been entirely successful. The earlier editions were successful because they were a mass of reliable and carefully checked observations. When complex ideas were included (rarely) they were carefully explained. The present edition has the same broad coverage, the same mass of data and observations (some unchanged from earlier editions), but it is no longer reliable. Mistakes appear on many pages, especially where new material has been added. Many of these errors are minor to be sure, but they are, nevertheless, misleading and greatly reduce the value of the book for the beginning student. For example, the caption for Fig. 15-2 (a P - T diagram for the polymorphs of Al_2SiO_5) mentions "phase boundaries indicated by short dashes" as being not experimentally determined, and "longer dashes" being an extrapolation of the kyanite-sillimanite curve. But all boundaries shown are solid lines and in addition the reference used (Morey, 1964) is hardly the most recent or most authoritative. Even if mistakes are corrected in later printings though, the book introduces so many ideas, concepts and ways of plotting data without adequate explanation, that it is no longer the easily accessible volume that earlier editions were. The volume can probably be used as a class text (and presumably will be, because it has a broader coverage of topics than any other English language introductory text), but it will require very careful checking by the instructor and a lot of back-up information. □

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Instrumental analysis

J.F. Coetzee

Instrumental Analysis. By Henry H. Bauer, Gary D. Christian and James E. O'Reilly. Pp.832. (Allyn and Bacon: Boston, Massachusetts, 1978.) \$23.95.

THE explosive growth of chemistry is making it increasingly difficult to decide which material should be included in the curriculum. It is also making it increasingly difficult for one or two authors to produce a uniformly authoritative textbook, particularly at the higher levels. This is certainly true of textbooks on instrumental analysis, because instrumentation is developing at an ever-increasing rate and this is occurring in a variety of essentially unrelated areas. The answer is to employ as many authors as are necessary to ensure authoritative treatment of all topics included. Any unnecessary proliferation of authors should be avoided, however, because it would create unnecessary editorial problems in achieving the reasonable consistency in approach, emphasis, style and other matters required for a pedagogically effective textbook. The compromise reached in the present text many not be ideal in that the 24 chapters have been contributed by 26 authors. Six chapters have two authors each, and only three chapters have been written by authors who have also contributed another chapter. Considering this plethora of authors, the undoubtedly odious editorial task has been performed well in most cases, however. Two introductory chapters, one to electrochemical methods and another to

spectroscopic methods, contribute significantly to the effectiveness of the text; a third, on separation theory, would have been a useful addition.

The balance of topics included in a textbook such as this must reflect to some extent the personal bias of the editors, but this reviewer finds it entirely reasonable. The three main subdivisions of instrumental analysis — spectroscopic, electrochemical and separation methods — are treated in approximately 350, 140 and 110 pages, respectively. Other topics make up the remaining 200 pages; among these are particularly useful chapters on computers and automation. An additional chapter, dealing with a critical comparison of trace analysis techniques, would have been welcome. The emphasis throughout is on instrumental methods of quantitative analysis, rather than on electronics and instrument design. No laboratory exercises are included. The rationale for these editorial decision to make the theoretical background of each method "as brief and qualitative as possible consistent with clarity and accuracy," but instructors who wish to strengthen these parts of the text can easily do so. An adequate number of questions and problems are included with the majority of chapters, but future editions of the text would benefit from the inclusion of a few more truly challenging problems and, of course, correction of some errors in the answers provided.

In summary, this text has major strengths and only comparatively minor weaknesses. It represents a new and basically sound departure in producing a textbook on instrumental methods of analysis, and it should be on the short list of anyone considering such a text. □

J.F. Coetzee is Professor of Chemistry at the Division of Analytical Chemistry, University of Pittsburgh, Pennsylvania.

General chemistry

Jerry A. Bell

Chemistry: An Introduction. Second edition. By Sydney B. Newell. Pp.563. (Little, Brown and Co.: Boston, 1980.) \$15.95. *Problem Exercises for General Chemistry.* By G.G. Long and F.C. Hentz Jr. Pp.364. (Wiley: New York, 1978.) \$9.80.

THIS is an outstanding, short text that fulfills the author's purpose, "to provide beginning students and their instructors with a comprehensive, understandable, and motivating introduction to chemistry."

The book begins with a simple, descriptive picture of the atom, to make the four succeeding chapters on stoichiometry,

naming and equations, comprehensible. It goes on to atomic structure, periodicity and chemical bonding in more detail as the background for material on states of matter and chemical change (solutions, equilibria, electrochemistry and rates) as well as brief introductions to nuclear, organic and biochemistry.

Although mathematics is used throughout, the text is suitable for students that have difficulty with quantitative reasoning; most concepts are introduced by homely and timely examples that involve relatively little mathematics and jargon, there are exceptionally clear appendices on working with numbers and units, and all examples are worked out in

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detail (by the factor-label method). Mathematics is treated as a tool and use of calculators is encouraged, especially when working with logarithms (pH). The end-of-chapter problems are grouped into two, roughly parallel sets and the solutions (set-ups shown) for the first set are given in an appendix; the rest of the solutions and suggestions for further problems, demonstrations, audiovisual aids, and so on are provided in an instructor's manual.

New terms are emphasised by italics and marginal notes in a second colour when they first appear; a very thorough index with a key to defined terms makes them easy to find later. Another feature is the appearance of a cartoon character, Maxwell's demon, in the figures. The demon is the author's "alter ego" and allows her to point out important features visually and informally, as many of us would like to do, if we could be present at our students' study. The demon is not gimmick, but an effective pedagogical device to which many students respond very favourably. Newell begins her first edition with the words, "I enjoy chemistry": students and instructors using her text will also.

The difficulties students have in solving problems generally fall into two categories:

interpretation and set-up. The second text is a self-study book that will give little help in interpretation.

The topics covered (in about 1,000 problems with answers) are the usual material of general chemistry: mole concept, stoichiometry, periodicity, atomic and molecular structure, phase properties, equilibria, thermodynamics, solutions, kinetics, and a bit of specific chemistry. (The material has also been used successfully in good high school classes.)

The authors' aim is to provide students with practice in working multiple choice problems to help them bridge the gap between the usual textbook problem statements and those they often meet in examinations. As the format and/or units of the answers are given in multiple choice problems, the student knows what she/he is working toward and is left 'only' the task of combining the information given in an appropriate way to get such a result.

The factor-label method is explained in concise, abstract form in Chapter 1 and then used consistently throughout the worked examples at the beginning of each chapter. Students who have trouble handling algebraic concepts will find the examples difficult to understand. Estimation (order of magnitude

calculation) of numerical results is emphasised as an aid to checking exact calculation and deciding whether a result is 'realistic'; often such an estimate is all that is required to select the correct response.

Occasionally, a bit more detail about the estimation made in the worked example would be welcome. The exact numerical solutions to the examples show what a student will actually see on a calculator followed by a discussion of precision, rounding-off, and significant figures; this approach is a very positive feature of the examples used. Significant figures to be reported in calculated values are explained both in terms of precision of the data and the more rote digit-counting procedure. The distinction between 'measured' and 'defined' values is made clearly in an appendix and applied throughout the text. The problems are straightforward, familiar and dull, that is, they provide no intrinsic stimulus for learning the material. Perhaps this is as it should be; this is a good practise book. The stimulus to practise must be provided by a teacher. □

Jerry A. Bell is Professor of Chemistry at Simmons College, Boston, Massachusetts.

Analytical chemistry

M.S. Greenberg

Analytical Chemistry. Second edition. By Donald J. Pietrzyk and Clyde W. Frank. Pp.700. (Academic: New York and London, 1979.) \$17.50. *Quantitative Analytical Chemistry*. Fourth edition. By James S. Fritz and George H. Schenk. Pp.661. (Allyn and Bacon: Boston, Massachusetts, 1979.) \$19.95.

ANALYTICAL CHEMISTRY by D.J. Pietrzyk and C.W. Frank is a revision of an earlier text and is intended for introductory courses in analytical chemistry, especially those shorter courses servicing chemistry majors and life-science majors. Other texts with this objective dilute the fundamental analytical chemistry to render the text 'relevant' by emphasising analytical applications. By concentrating on fundamental principles of modern chemical analysis and illustrating these principles with applications to chemical, environmental, clinical and pharmaceutical problems, the authors provide the student, with some exceptions, with an excellent introduction to the theory and practice of analytical chemistry.

After a core of six chapters dealing with

prerequisite concepts, the text emphasises five major areas: volumetric methods, potentiometry, spectroscopy, chromatography and electrolysis methods. These chapters are followed by a chapter on experimental techniques and 41 laboratory experiments.

The core chapters include discussions of the nature of analytical chemistry, the analytical method, stoichiometry, statistics, operations in analytical chemistry and chemical reactions. These chapters are well written and very informative about the nature and role of analytical chemistry. However, some important concepts are not pursued in reasonable detail. The statistics chapter introduces the t statistic and confidence limits, but does not demonstrate their use in significance testing. Pronouncing that "the activity concept is an insignificant one" is bothersome. The introductory student should be aware of thermodynamic versus conditional equilibrium constants. Later, the authors note that since ion-selective electrodes respond to ion activity, ionic strength must be held constant to relate potential to concentration. However, the text does not demonstrate the calculation of or relationship between ionic strength and the activity coefficient.

The chapters dealing with volumetric methods of analysis are the best in the text. The authors show the student a chemical approach rather than a formula approach to problem solving — important equilibria are identified, equilibrium constants

written, chemically reasonable, simplifying assumptions are made and the problem solved. Further, the order of the chapters lends clarity to the material. For example, precipitation methods are divided into chapters on gravimetric methods and precipitation titrations, with the latter following the chapter on potentiometry. The chapters on redox methods and ion-selective electrodes are preceded by an excellent chapter discussing conventional cell notation and the Nernst equation. Tables summarising species determined and reagents and conditions for analysis by each method are also presented.

The remaining chapters on instrumental methods of analysis are all descriptive and contemporary. However, the emphasis on fundamentals, so evident in the earlier parts of the text, is missing. Deviations from Beer's law, their sources, elimination and implication in instrument design are not discussed. Instrument components and configuration (single beam, double beam) are not pursued with enthusiasm. On the other hand, differences in configuration for molecular versus atomic absorption instruments are pointed out. Chromatographic methods are developed without discussions of plate and/or rate theory and the Van Deemter equation. Electrolytic methods are introduced but no mention is made of the important new voltammetric methods.

The experiments are those which are done in most courses in analytical

chemistry: analysis of well characterised unknowns. The experiments are presented in a cookbook style, so the instructor may wish to supplement with assigned reading or lectures.

Despite the descriptive nature of the instrumental analysis chapters, this text is one of the best for teaching introductory analytical chemistry. The instructor may wish to supplement the instrumental analysis chapters with more theoretical concepts as noted, but the lucid writing and excellent organisation of this text teach the student to think analytically.

In revising the third edition, the authors of *Quantitative Analytical Chemistry* attempted not only to update the text, but also to make explanations clearer and difficult concepts more understandable. This revision is a significant improvement, and should maintain the popularity of the earlier texts in this series.

The text is intended both for short, introductory courses for chemistry majors and students in related disciplines and for more intensive courses for chemistry majors. The text is arranged in two sections: Part I, Principles and Theory, and Part II, Laboratory Techniques and Procedures. The authors recommend the first 14 chapters for a short course stressing the fundamentals of analytical chemistry, with the more specialised, 11 remaining chapters added for a more modern course in chemical analysis.

As a result of the dual audience objective, the chapter organisation in Part I is not ideal. After some introductory chapters, most texts present discussions of volumetric methods of analysis followed by chapters on instrumental methods. After the expected chapters on the nature of analytical chemistry, the analytical method and statistics, two chapters on gravimetric and spectrophotometric methods are presented. Overview chapters on volumetric methods and chemical equilibria follow immediately, then the theory and applications of aqueous and non-aqueous acid-base, precipitation, complexation and oxidation-reduction methods are presented in seven chapters. The instructor may wish to rearrange the middle chapters, especially moving the spectroscopy chapter to the end. The order of the more advanced chapters on instrumental methods is traditional.

As noted, the revised and new material have significantly improved the text. The statistics chapter is very good, yet a discussion of the use of confidence limits for significance testing would be expected for a majors text. The addition of worked problems in complexation chemistry lends clarity to that discussion. Chapter 5, Spectrophotometric Methods of Analysis, presents an excellent introductory discussion of Beer's law and chemical instrumentation. The best chapters of the text are those dealing with instrumental methods. The reorganised discussion of electroanalytical methods is followed by an

excellent chapter on potentiometry with ion-selective electrodes. As promised in the preface, the chromatography unit is strong, probably the best available in an introductory text. The chapters on atomic and molecular spectroscopy emphasise fundamental principles of spectroscopy and instrumentation, building on concepts presented in Chapter 5.

However, the "new treatment" applied to the presentation of acid-base chemistry was most disappointing. Approximations employed for pH calculations of weak acids and bases are not carefully justified. Although weak acids do ionise to a small degree such that the analytical and equilibrium concentrations of protonated acid will be nearly equal, the assumption is not justified or checked after the calculation. Further, implications of ignoring the autoprotolysis of water are not explored. For diprotic acids, the text states that if $K_{A1} \gg 100 K_{A2}$, the second dissociation may be ignored in pH calculations. Though this approximation is reasonable, it is not carefully justified. While the authors' intention to approach pH calculations by identifying the principal equilibrium and rearranging the

appropriate equilibrium constants is desirable, their presentation is not sufficient. On the other hand, their discussions on feasibility of titrations, indicators and the new chapter on non-aqueous acid-base titrations are good.

Part II is an acceptable laboratory manual. Experiments are grouped into units on analytical operations, gravimetric procedures, volumetric glassware, titrimetric, spectrophotometric, electro-analytical and separation procedures. Each experiment begins with a brief theoretical discussion followed by the procedure and concludes with questions. The experiments are those which characterise most teaching laboratories.

This text, though awkward in chapter progression, will function well in a chemistry majors course in modern chemical analysis. While it would be possible to use this text in a short introductory course, texts written specifically for those courses should be considered also. □

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General chemistry texts

R.O. Ragsdale

General Chemistry/Principles and Structure. Second edition. By James Brady and Gerard Humiston. Pp.786. (Wiley: New York and Chichester, UK, 1978.) \$19.95. *Laboratory Manual for General Chemistry Principles and Structure*. By Jo Beran and James Brady. Pp.520. (Wiley: New York and Chichester, UK, 1978.) \$8.95. *Basic College Chemistry*. By Don Roach and Edmund Leddy Jr. Pp.636. (McGraw-Hill: New York, 1979.) \$16.95.

THE text *General Chemistry, Principles and Structure*, by J.E. Brady and G.E. Humiston is a successful entry in a highly competitive market. Joint efforts between authors and publishers have resulted in a number of excellent texts at the level of Brady and Humiston. Some of them are: *A Conceptual Approach, Chemistry* by C.E. Mortimer (Van Nostrand: New York and London, 1979), *Chemistry, the Central Science* by T.L. Brown and H.E. Le May Jr (Prentice-Hall, Englewood Cliffs, New Jersey, 1977), and *Chemical Principles* by W.L. Masterton and E.J. Slowinski (W.B. Saunders: London and Philadelphia, 1977).

The Brady and Humiston text stands out alone with its unique feature of stereo

illustrations. The stereodiagrams as well as the other illustrations are all well done. I have been successful in seeing all of the stereodiagrams in 3-D. All of the stereodiagrams are not necessary and in many cases students would be better off making their own models.

The text is well organised and well written. It has ample questions and problems. The second chapter, which introduces stoichiometry, has 19 examples and 63 questions and problems at the end of the chapter. The chapter on acid-base equilibria in aqueous solution has 15 examples and 71 questions and problems.

This is a traditional freshman general chemistry text for science and engineering majors. It is intended for both students who have had high school chemistry as well as those who have not. This text is similar to those mentioned above in using a theory and principles approach to the teaching of chemistry. Like the other texts it has a limited amount of descriptive chemistry. It has three chapters on representative metals, nonmetals and metalloids, and transition elements.

The freshman students in the US are probably at the same level as the advanced level students in the UK. The UK students are exposed to much more organic chemistry than their US counterparts. There has been a resistance to texts which offer much more than one chapter of organic chemistry. This is a paradox since many of these students will not be taking any more chemistry. This resistance to organic is also true for descriptive chemistry.

The Brady and Humiston text is very readable and the second edition seems to be an improvement over the successful first edition. Textbook authors are slowly going to the SI units. This text makes use of both SI units and the metric system. It will probably be some time before there is complete conversion to the SI system.

Most successful US books now come with a complete teaching package and Brady and Humiston is no exception. There is a student study guide available as well as a laboratory manual. The book, *Laboratory Manual for General Chemistry Principles and Structure* by J.A. Beran and J.E. Brady, has been designed to accompany the textbook. This manual is also suitable for a number of general chemistry texts.

The laboratory manual contains both quantitative and qualitative experiments (altogether 44 experiments). The emphasis on organic chemistry in the US general chemistry is reflected in the laboratory manual as 2 out of 44 experiments involve organic chemistry. There is a lack of emphasis on accuracy and precision in measurement in the manual.

In the first part of the manual 18 laboratory techniques are illustrated by actual photographs whose reproduction in the text are of high quality. For beginning students these instructions will be invaluable. The authors do have an

annoying habit of continually referring to these techniques. One would hope that the students would be able to refer back to the techniques themselves instead of the cookbook instructions (. . . "remove copper from the rubber policeman . . . (Technique 6)" and " . . . heat the copper metal to dryness with a Bunsen burner (Technique 9(a))". The experiments all have a report sheet with questions and a pre-laboratory assignment sheet with questions and problems.

There are some interesting experiments. The law of multiple proportions is demonstrated by starting with copper (II) bromide and converting it to copper (I) bromide. Copper is obtained by displacement with magnesium. A limiting reactant experiment is achieved by taking an unknown mixture of the salts $\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$ and $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$ and adding water to them. Barium phosphate precipitates and one determines the limiting reactant and the composition of the salt mixture.

The third book that I reviewed left me in a quandary as to the students for whom it was intended. The text, *Basic Chemistry* by D. Roach and E. Leddy Jr, has a table of contents which is similar to texts that are used for science major courses. The authors give no indication in the preface for whom it was written. After perusal of the text, I came to the conclusion that it was

written for nurses and students in the allied health fields as the text is definitely a full level below Brady and Humiston. Some texts which are similar in level are *Foundations of College Chemistry* by Hein (Dickenson: Encino, California, 1977), *Basic College Chemistry* by Mitchell (Harper and Row: New York, 1978) and *Introduction to Chemistry* by Dickson (Wiley: New York and Chichester, UK, 1979). Roach and Leddy's text is longer than the ones mentioned above by some 100 pages. Many nurses' courses do general chemistry for one semester and in some cases only one quarter. The rest of the year is spent on organic and biochemistry. If the book is intended for this audience it has perhaps missed the mark.

It is conceivable that junior college and community college students might spend a year on a low level course. If these students then decided to go along the science major route they would need to take a Brady and Humiston type course.

Roach and Leddy's book uses a definition approach to chemistry. This approach makes the text seem quite sterile. The more difficult problems are starred. One wonders when a problem such as '4.21 g/ml = ? g/liter' is starred. □

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Soil chemistry

P.H. Nye

Soil Chemistry. A. Basic Elements. Edited by G.H. Bolt and M.H.M. Bruggenwert. Pp.281. (Elsevier: Amsterdam, Oxford and New York, 1978.) \$19.95. *B. Physico-Chemical Models.* Edited by G.H. Bolt. Pp.479. (Elsevier: Amsterdam, Oxford and New York, 1979.) \$73.25.

THESE two volumes have been largely written by Professor Bolt and his colleagues at the Agricultural University of Wageningen, The Netherlands. Part A (*Basic Elements*) is intended as a textbook for advanced undergraduates. Part B (*Physico-Chemical Models*) elaborates the theoretical material of Part A. Both parts concentrate on physicochemical aspects, and a general title such as "Soil Physical Chemistry" would have been more appropriate than *Soil Chemistry*.

The strength of Part A lies in its sound theoretical development, its sensible ordering of topics, its uniform symbolism and terminology in spite of nine authors, and the numerical examples at the ends of the chapters. There is no other textbook

covering the same ground at an advanced level.

The first chapter on the composition of the soil, and the second, a potted account of chemical, especially solubility, equilibria summarise the background anyone who hopes to understand the book must have. A fairly intelligible account of double layer theory and cation adsorption and exchange follows. The chapter on solubility equilibria in soils, including redox reactions involving iron, which are treated in terms of electron activity pe , is good. Chapter 7, "Transport and Accumulation of Soluble Soil Components", is often obscure (figure 7.1, for example, and the phrase "autonomous flux of solute"), though the distinction between self-sharpening and diffuse boundaries in solute displacement is well presented. These theoretical treatments are then used by Van Beeman and Brinkman to discuss soil formation in a way that achieves the right balance between ideal equilibrium and real non-equilibrium behaviour. The application of theory to saline and sodic soils is well presented. The volume concludes with an interesting account of soil pollution, which contains much more descriptive material than elsewhere in the book. We learn, for instance, that 60–90% of the deaths of street trees in Dutch town centres are caused by gas leaks following conversion to

natural gas. This section should convince students that the theory they have been invited to master in previous chapters is essential to understanding the fate of the great variety of pollutants now being dumped on the soil.

This second edition has eliminated many of the errors and 'Dutchisms' that occurred in the first edition. Unfortunately, the term "Free Enthalpy" is perversely retained throughout for Gibbs free energy G , without explanation. The uncertain student, having learnt to make this mental switch, will be mystified to read on p.14 that "to each chemical species in a reaction mixture a certain amount of ('free') energy can be ascribed." The phraseology is often awkward and some curiosities remain, such as a "gift" of irrigation water. It is a pity that the opportunity was not taken to have the text revised by an English writer.

Part B is considerably more difficult than Part A. The first half attempts to explain the relation between the proportions of exchangeable cations adsorbed on negatively charged clay and soil surfaces and their proportions in an equilibrium solution. Chapters 1, 2 and 3 by Bolt develop from first principles the theory of the diffuse double layer, the thermodynamics of cation exchange and the distribution of cations of differing charge in the electric field created by uniformly charged negative surfaces.

Much of this is Professor Bolt's own contribution to the subject, and it is good to have it collected together. The theory developed has limited application because it assumes the surface charge is uniform. In reality, the negative charges are discrete, so it is gratifying that Chapter 4 by Harmsen deals specifically with discrete site models, leading to a full account of the application of Guggenheim's theory of mixing to cation exchange equations. Diffuse double layer theory does not enable one to decide whether the selectivity coefficient in a heterovalent exchange (for example, $K^+ - Ca^{2+}$) is more likely to be constant if the activity of an adsorbed cation is represented by its equivalent fraction (Gaines and Thomas equation) or its mole fraction (Vanselow equation). The conclusion reached from mixing theory is that the selectivity coefficient should always lie between these two extremes. Up to this point Chapter 4 is well worth the effort; it then gets lost in remote realms of speculation, assuming various arrangements of discrete sites.

We are brought back to earth in Chapter 5. Bruggenwert and Kamphorst tabulate in uniform fashion thermodynamic data on 350 cation exchange reactions on clays, 265 on soils and a further 119 for heavy metals on soils, the last providing useful evidence of the high affinity of soil for these elements at low surface coverage; otherwise, they are able to draw disappointingly few conclusions from their mammoth collection of data.

In Chapter 6, Maes and Cremers consider the effect of surface charge density on ion selectivity in clays. In the exchange between mono- and divalent

ions, they show that a simple electrostatic model is only partially successful because of ion-solvent interactions that are difficult to calculate. The corresponding discussion of exchange between homovalent ions (sections 6.1.3 and 6.1.4) is marred by obscure and slipshod writing, and the accompanying tables (numbers 6.3 and 6.4) omit to mention the ions for which data are given. The greatly increased stability of ion complexes, such as copper-ethylenediamine, when adsorbed on clay surfaces is clearly described.

In a valuable account of anion exclusion from negatively charged surfaces, Bolt and de Haan suggest that the apparent reduction of chloride exclusion from the surface of calcium montmorillonite is caused by plate condensation rather than ion pair formation as suggested by Edwards, Posner and Quirk. This controversy badly needs settling by further experimental work.

A highly compressed chapter on the interaction of orthophosphate ions with soil lacks critical insight into the complex reactions described. If adsorbed anions were not to be more comprehensively covered — and phosphate is the only anion considered — this chapter might have been omitted.

The last third of the book deals with kinetics. Bolt treats movement of electrolytes in artificially packed soil columns in terms of advanced chromatography theory, and the few who will stay the course will be rewarded by a "guideline for guessing" the development of displacement fronts at the end of the chapter. Van Genuchten and Clearey extend this treatment to undisturbed

profiles, and show lucidly that a model based on zones of mobile and immobile water has marked success. Bolt and Groonevelt's chapter (Chapter 11) on electrokinetic phenomena in soil is the best account I have read and is neatly unified in terms of the thermodynamics of irreversible processes. The concluding section on the conductivity of clay suspensions reveals that exchangeable ions on clays have considerable surface mobility (p. 422), yet the treatment of diffusion effects in soil columns (p. 299) implicitly assumes that exchangeable ions are immobile. This inconsistency is not discussed. In fact, exchangeable ions in soils do have very different mobilities from exchangeable ions in clays, and the reasons for this important difference should have been referred to.

The book concludes with a suggestive account by Brinkman of clay transformations, in which the equilibrium and kinetic ideas developed in earlier chapters are applied to the long-term processes of soil formation. An advanced text on soil development from this viewpoint is badly needed and since good field men with an equally good understanding of physical chemistry are rare, perhaps Brinkman could be encouraged to write it.

This book should certainly be acquired by soil science libraries, and by wealthy workers in the fields of ion exchange and solute movement in porous media. □

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How to know

Almut G. Jones

How to Know the Grasses. Third edition. By Richard W. Pohl. Pp.200. (Brown: Dubuque, Iowa, 1978.) Clothbound \$8.95; paperbound \$6.95. *How to Know the Mosses and Liverworts.* Second edition. By Henry S. Conard and Paul L. Redfearn Jr. Pp.302. (Brown: Dubuque, Iowa, 1979.) Clothbound \$8.95; paperbound \$6.95. *How to Know the Lichens.* Second edition. By Mason E. Hale. Pp.246. (Brown: Dubuque, Iowa, 1979.) Clothbound \$8.95; paperbound \$6.95. *How To Know the Seed Plants.* By Arthur Cronquist. Pp.153. (Brown: Dubuque, Iowa, 1979.) Clothbound \$8.95; paperbound \$6.95. *How to Know the Ferns and Fern Allies.* By John T. Mickel. Pp.229. (Brown: Dubuque, Iowa, 1979.) Clothbound \$8.95; paperbound \$6.95.

THE five volumes of the popular, economically priced, *How to Know* series reviewed here have certain features of format and organisation in common upon which I wish to comment beforehand. They are intended to serve the amateur naturalist and the serious student, as well as the professional scientist. In contrast to the pocketbook format of earlier editions (preferred by me for the books intended as field guides), they are of notebook size (19×23 cm). The paperback versions have a spiral-coil back, and a rather frangible cardboard cover not very suitable for the rigours of fieldwork. Following the instructive introductory chapters, the body of each book is comprised of abundantly illustrated keys. The final pages are assigned to a combined index and, usually pictured, glossary. In my opinion, ease of finding would be enhanced if these last two items were separated.

As the two earlier versions of *How to Know the Grasses* were authored by the same renowned agrostologist (R. W. Pohl), the reader would expect an expertly written

and polished product in this third edition. He will not be disappointed. The booklet includes keys, illustrations and distribution maps for 324 of the most common grasses of the US. Most of the meticulous drawings were done by the author himself. Following the system of classification published by Stebbins and Crampton in 1961, six subfamilies are recognised. Chapters of the introduction are devoted to definitions of structural terms. A key is provided distinguishing grasses from sedges and rushes. The student is instructed on how to collect and prepare scientific specimens. A list of 29 references suggests supplementary literature. Preceding the artificial keys to genera and species is a chapter on recognising the grass tribes and a preliminary key to major groups. Although clearly written for the beginner, the book will also appeal to the seasoned naturalist and the professional plant systematist. It is an excellent field and laboratory guide and a must for any student of grasses.

Billed as the second, this is actually the

third edition of this handy guide to the North American mosses and liverworts, *How to Know the Mosses and Liverworts* (by H.S. Conard and P.L. Redfearn Jr). While the amateur will be overwhelmed with the variety of forms and hidden structural beauty of these unobtrusive flowerless plants, the professional botanist will be impressed with the careful detail shown in the illustrations, as well as the endeavour for scientific accuracy apparent in the organisation of the contents. Introductory chapters deal with life cycles, diagnostic characteristics, habitats, preparation of specimens, and classification of categories above the level of family. Approximately 1,700 species of bryophytes occur in North America, and all the common ones can be identified using this booklet. Since the previous edition, 112 species and 42 genera have been added. The strictly dichotomous keys are broken up so that the user first arrives at the generic name and then finds the species under each genus. A brief descriptive paragraph is provided for each species. As recent taxonomic revisions have resulted in a considerable number of name changes, a list of synonyms is included. A bibliography of 35 entries inspires background and supplementary reading. The book is a treasure as a field guide, and it is definitely orientated to be a text in an introductory bryology course.

How to Know the Lichens by M.E. Hale is an attractive field and laboratory guide to the identification of lichens. It is a revised and an enlarged version of the first edition prepared, after an interval of 10 years, by the same author. There is little difference between the two editions in general outline and organisation. The geographical region considered is the North American continent but, as many lichens have a circumboreal distribution, the keys are largely applicable to the lichen flora of the entire Northern Hemisphere. Seventy new entries have been added since the first edition, bringing the total to 427. Only foliose, fruticose and squamulose lichens are treated, the crustose forms having been omitted "because they are too numerous and too little known". In the introduction, the student is informed about the symbiotic nature of lichens, about their economic uses and their sensitivity to air pollution. Several well illustrated pages are devoted to chemical, colour and crystal tests which are so essential for the identification of lichens. The student is further instructed in the use of technical terminology and in methods for storage and culture of specimens. A list of 37 references gives sources for additional information. All keys are dichotomous and conveniently broken up. Keys for the main sections are preceded by alphabetic lists of genera with a citation of their salient features. In addition to descriptions and illustrations for the major species, inset maps indicate their ranges of distribution. The keys are followed by a list

of synonyms and a classification of genera and species. The booklet constitutes an authoritative text well suited for any course on lichen systematics.

How to Know the Seed Plants by Arthur Cronquist, is a successor to the section on seed plants in H.E. Jaques' *Plant Families: How to Know Them* (1949). Approximately 375 families of seed plants are recognised and those represented by plants growing spontaneously or frequently cultivated in the US are treated in the dichotomous keys. Each family is identified by both vernacular and scientific names. In addition to a description, each entry gives information on common genera and on geographical distribution. One or more of the characteristic species are illustrated. Whereas I consider the booklet a worthwhile addition to my botanical library, I doubt whether the uninitiated amateur, who is so obviously addressed in the rather perfunctory introduction, will find it all that attractive. Amateurs have generic concepts; they may think, for example, in terms of cottonwood, lilac, clover, carnation, and so on, but would not ordinarily view these plants as members of Salicaceae, Oleaceae, Fabaceae, and so on. The book is not, nor intended to be, a substitute for an amateur's field guide. In my opinion, the introduction is aimed at too low a level of scholarship, and falls short of giving adequate background and technical instruction to potential readers who really would appreciate a text with pictured keys to the families of seed plants, namely college students and their instructors, as well as reasonably well informed naturalists. The brief chapter on principles of classification and nomenclature does not do justice to either the excellent keys or the phylogenetic outline of classification. The omission of a bibliography on background and supplementary literature is hardly forgivable and definitely impairs suitability

of the book for college-level courses on plant identification and systematics.

It is an inspiration to leaf through a scholarly prepared text like *How to Know the Ferns and Fern Allies*, by John T. Mickel. The volume succeeds in fulfilling the need for a modern field guide to all the North American petridophytes. To accommodate the amateur, technical terminology has been minimised, but without sacrificing clarity and accuracy. As emphasis rests on identification, family names are avoided and, within the major groups, the plants are keyed directly to the generic names. Species are treated in secondary keys under the alphabetically arranged genera. Identification is aided by diagnostic drawings, as well as inset maps indicating geographical distribution. Relationships among North American fern genera are presented diagrammatically at the beginning of the text. Several chapters preceding the keys furnish a comprehensive introduction to definitions of structural characteristics, life cycles, hybridisation and cytological phenomena, techniques for spore culture, as well as instruction on how to establish a fern garden and how to prepare herbarium specimens. Valuable bibliographical references are given not only at the end of some chapters, but also in the form of a list of 54 state and regional identification manuals. Preceding the index and glossary is a checklist of North American ferns and fern allies. The book is highly recommendable as a text in college courses at any level; it will also be regarded as a valuable reference work by naturalists and professional scientists dealing with the vascular cryptogams. □

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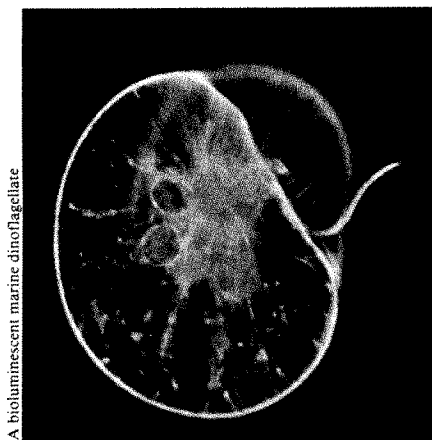
Introduction to phycology

M. G. Kelly

Introductory Phycology. By F. R. Trainor. Pp.525. (Wiley: New York and Chichester, UK, 1978.) \$21.50. *Biology of Seaweeds/Levels of Organization*. By A. R. O. Chapman. Pp.134. (University Park Press: Baltimore, 1979.) \$10.75. *Introduction to the Algae: Structure and Function*. By Harold Bold and Michael Wynne. Pp.706. (Prentice-Hall: Englewood Cliffs, New Jersey, 1978.) \$36.40.

ALGAE are responsible for most of the energy input to marine and freshwater communities. They play a major role in controlling water chemistry, especially of oxygen, pH and nutrients. The types of algae in a water body often determine the species composition of the higher trophic levels. Algal productivity controls the locations of the world's major oceanic fisheries. In lakes, too few algae mean a poor fishery sport while too many mean extreme water quality problems. It can be easily argued that algae are fully as important as the land plants.

As Trainor points out in *Introductory Phycology*, these factors have led to an increased interest in algae, not only on the part of biologists, but also by water quality



A bioluminescent marine dinoflagellate

From *Biology* by Helena Curtis, reviewed on page 106

engineers, geochemists and others. This increased interest has produced a need for modern texts on phycology: these three respond to that need.

The shortest of these books, *Biology of Seaweeds/Levels of Organization* by Chapman, is the most specialised. Although it deals only with the macroscopic algae of coastal areas, it provides much basic information for a general algology course. The other two texts are comprehensive, in that they cover all of the phylogenetic groups. They are also 'classical' in approach, proceeding group by group, emphasising the taxonomy, morphology, reproduction, and to a much lesser degree physiology, biochemistry, ecology and distribution. Chapman, on the other hand, proceeds on the basis of levels of organisation, progressing from cellular ultrastructure through the ecology of communities. I find Chapman's approach much more appealing. It provides a holistic understanding of 'how algae work', that is, how they are adapted to exist and function within an environment. The book emphasises ideas and leads a student's train of thought within an organised framework. The approach of the other two books leads to rote memorisation and a fragmentation of ideas which students (and thoughtful professors) may find less than appealing. On the other hand, the book by Trainor and *Introduction to the Algae: Structure and Function* by Bold and Wynne are organised in the way many algology courses are taught, and this alone will probably increase their acceptance.

All three texts differ in the way algae are divided into divisions and classes. Trainor uses five divisions, and Bold and Wynne use nine. Chapman discusses three (those containing marine macroalgae), and simply avoids mentioning the controversies of classification by usually referring simply to red, brown and green algae. His approach seems most sensible. Why teach a scheme of classification and set of terminology that authorities disagree upon and that will probably be completely revised in a few years? Bold and Wynne and also Trainor recognise this problem. The former state "... nature mocks human categories' and this certainly seems

to be the case when one compares the more or less current systems of classification ...". Trainor quotes Dodge as stating that classes "... are the only stable feature which has persisted through the numerous classification schemes of the past few years". Perhaps both books should have followed Dodge's suggestion — don't confuse students by using conflicting classification schemes at the division level.

The two classically organised texts, Trainor, and Bold and Wynne, are very different in the amount of detail presented. Trainor is sparsely referenced and reads like transcripts from a set of very lucid lectures. Wynne and Bold, on the other hand, include 87 pages of references and the text, in places, reads like a monograph. Both provide generic examples, but Trainor's are often very sketchy while Bold and Wynne's are usually too detailed for teaching use. The difference is illustrated by the discussion of the genus *Hormotilopsis*, a somewhat obscure green soil alga first described by both Trainor and Bold. Both texts refer to it. Trainor uses it as an example of dispersion of unicells derived from planospores, but says nothing else about it so that it remains an unassociated Latin name in a student's mind. Bold and Wynne, on the other hand, feel they must tell the student that it is distinguished from *Hormotila* by having quadriflagellate rather than biflagellate zoospores, provide several illustrations, and tell when and by whom it was first isolated. I think most algologists would agree that a student would not be deprived if he never heard of either genus.

Chapman's book, published by a fairly small firm, contains few, if any errors in composition and editing. The other two, published by major houses, contain major errors. For example, in Bold and Wynne a block of text is missing on page 5. In Trainor figures 17-6 and 17-7 are identical. The caption for one states it shows algae washed ashore after a storm, while the other is supposed to be the intertidal of the Arctic; reproduction of the illustrations was so poor I was unable to tell which, if either, was shown.

A major reason for the increase of interest in algae is recognition of the importance of their ecology. Bold and Wynne deliberately avoid this topic, Trainor includes material, but much of it is solely descriptive and out of date, and nearly all would be covered in an introductory ecology course. Chapman, within his limited scope, provides an outstanding and stimulating discussion.

In summary, I will recommend Trainor to a student who wants to read about algae without taking a formal course. Bold and Wynne will become a well worn member of my reference shelf and will be owned by all of my graduate students. Finally, I look forward to someone writing a text with the organisation, style and balance of coverage that Chapman has but which covers all of the important groups. □

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Entomologically speaking

R.W. Thorp

How to Know the Insects. Third edition. By Roger Bland and H.E. Jaques. Pp.409. (Brown: Dubuque, Iowa, 1978.) \$7.95. *How to Know the True Bugs*. By J.A. Slater and R.M. Baranowski. Pp.256. (Brown: Dubuque, Iowa, 1978.) \$6.95. *How to Know the Spiders*. Third edition. By B.J. Kaston. Pp.272. (Brown: Dubuque, Iowa, 1978.) \$6.95. *How to Know the Mites and Ticks*. By Burruss McDaniel. Pp.335. (Brown: Dubuque, Iowa, 1979.) \$7.95.

Two new volumes and two revised third editions dealing with insects and arachnids have been published in the *How to Know* series since 1978. All four have a plate size 2×4.5 cm larger than earlier editions with an easier to read double column format. One problem with the volumes on true bugs, spiders, and especially mites and

ticks is that descriptions of genera, species and keys to species are inserted among the couplets of the keys to genera, occasionally separating halves of these couplets by up to two pages. Since many of the taxa treated in these volumes are cosmopolitan, they may prove useful as identification manuals in other countries.

In most particulars, the edition of *How to Know the Insects* by R.G. Bland and H.E. Jaques is a vast improvement over the second edition: more than half again as many families are keyed; families of scale insects (Coccoidea) are not keyed, but are discussed and diagnosed; family discussions include more complete descriptive and biological data; many subfamilies of large families of Orthoptera (Acrididae, Tetigoniidae, Gryllidae), Coleoptera (Scarabaeidae, Chrysomelidae, Cerambycidae, Curculionidae), Lepidoptera (Saturniidae, Arctiidae, Pyralidae) and Hymenoptera (Formicidae, Vespidae, Sphecidae) are diagnosed and discussed, although keys are not provided; there are many more illustrations and their larger size, improved quality and detail make them much more useful; the classifications are more current and orders and

families are organised in a more closely phylogenetic sequence; although illustrations are not included in the terminal Index and Glossary, references to appropriate text figures are found with each term defined. Missing are the useful systematic lists of families of North American insects for each order. Also gone are the folksy humour and enthusiasm of the initiator of the series, Professor Jaques, but these are replaced by more precise and erudite discussions of the diversity and interest of insects.

Introductory chapters on how to collect, preserve, mount, curate, observe, rear and where to look for insects are concise and well done. The chapter on structure and development is brief, but does seem to cover the bare essentials of structure necessary to use the keys. The keys are well constructed, with a minimum of technical jargon allowing easy use even by a novice. A key to orders of adult insects is followed by treatments of each order.

Each order is introduced with a brief descriptive diagnosis, habits, life histories, habitats occupied, special collecting and curating techniques, and estimates of the numbers of species and families in North America and species worldwide. This is followed by a key to the common families of the order. The family key is followed by brief structural diagnoses of each family, their principal habitats and often by brief descriptions of the most common species. A list of general references usually concludes each ordinal treatment.

Few errors of any import were noted. The reference to one of the best new textbooks on entomology by H.V. Daly, J.T. Doyen and P.R. Ehrlich, *An Introduction to Insect Biology and Diversity* (McGraw-Hill: New York, 1978), is curiously included within the reference to the book by M.D. Atkins. Missing from the same list of general references in insect biology is another important multi-authored book edited by D.F. Waterhouse (*The Insects of Australia*. Sponsored by CSIRO, Canberra. Melbourne University Press, 1970). Although the key to Mecoptera families correctly states that Bittacidae have only a single large claw on each tarsus, figure 223A clearly shows two tarsal claws on each leg of *Bittacus apicalis* Hagen.

The quality of the illustrations in this volume is not on a par with those in the better known field guides. However, it does provide keys for identification which the field guides usually do not and the keys are simpler and easier to use than those of the more expensive general texts. Although this volume lacks lists of family group and higher classificatory taxa for each order, its coverage of over 60% of the families of America north of Mexico, its clear, concise, accurate keys and descriptions, and its relatively low price make it an attractive alternative to the texts for field and laboratory courses devoted to identification and classification of adult insects.

How to Know the True Bugs by J.A. Slater and R.M. Baranowski is an excellent addition to the series, and includes keys to the families of Hemiptera-Heteroptera of North America, to 475 genera, and to 65 species and 2 subspecies in 10 of these genera. In addition to the usual introductory material on life history and morphology, and collecting, preserving and labelling methods, a special historical section introducing early European and American hemipterists is included. Following the key to families based on adult Hemiptera is a separate key to families based on nymphs. The majority of the book consists of family treatments introduced by brief descriptions and biological information, most followed by a key to genera with brief discussions of each genus often followed by descriptions of common species. Most of the family treatments end with references to pertinent literature. At the end of the book is a list of common names of true bugs as approved by the Entomological Society of America, followed by the standard index and glossary.

Few errors of any import were found. In the key to genera of Coreidae the genus *Thasus* was apparently omitted from the first half of couplet 2. The illustrations are of excellent quality. The keys are clear, concise and easy to use. This volume will be useful to all who have occasion to identify the genera true bugs. I know of no other single reference which serves this need for all of North America.

The larger format of the new edition of *How to Know the Spiders* by B.J. Kaston has increased the size of the illustrations and improved their clarity, quality and usefulness, especially those showing webs and snares. More than 40 new figures have been added, and the cartoons of the second edition have been deleted. A new chapter presenting a pictured key to the orders of Arachnida increases the utility of this volume, and places spiders in the context of their closest relatives. One of the more useful features of the second edition which has been carried forward into the current edition is the list of families of spiders arranged in their higher classification groupings and with footnotes denoting families which have representatives in America north of Mexico. The bulk of the book is devoted to descriptions of the families and keys to the more common genera. The additional genera and species treated in this volume bring the coverage to nearly double that of the original volume. Since nine families are uncommonly collected, they are only briefly discussed at the point where they come out in the key to families and therefore do not appear in the list of families in the table of contents.

The volume could be tightened up a bit by eliminating repetition of several figures, some of which are reprinted on four separate pages (for example, *Atypus niger* ventral view is used for Figs 26, 49, 63, 160), where a single figure with subsequent

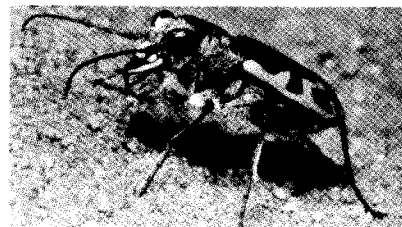
references to it would suffice. Nonetheless, this volume remains the best resource for identification of North American spiders.

McDaniel should be complimented on his attempt to produce in *How to Know the Mites and Ticks* an identification manual for such a taxonomically difficult group. Especially creditable is the key to families and genera of the Oribatei. Most of the figures are well done and useful. However, figures 1-3 in the section on morphology could stand enlargement and the captions are missing for some (for example, 12, 13, 18, 25, 99-102, 113) or separated by a column or even a page from the figure they belong to. Hypopi of astigmatid families (for example, Acaridae, Anonitidae and Glycyphagidae) are figured and some are diagnosed.

This volume would benefit from the inclusion of a list giving the higher classification (order to families) rather than referring the reader to the excellent manual by G.W. Krantz (*A Manual of Acarology*, 2nd edition, OSU Book Stores: Corvallis, Oregon, 1978). This volume suffers from uneven treatment of taxa with considerable space devoted to such families as Pyemotidae, Chyletidae and Listrophoridae while economically and medically important large families such as Eriophyidae, Tetranychidae and Trombiculidae have only one species treated. The so-called keys to genera of many families are not dichotomous keys with contrasting couplets, but rather a sequential series of generic diagnoses (for example, Spinturnicidae, Tarsonemidae, Pyemotidae, Chyletidae, Tenuiplapidae, Stigmaeidae, Tydeidae, Saproglyphidae, Psoroptidae, Glycyphagidae, Anonitidae, Pyroglyphidae, Listrophoridae, Sarcopidae, Epidermoptidae, Chirodiscidae, Myocoptidae). The key to species of *Ornithodoros* should be set in a smaller typeface with different couple designations so as to distinguish it from the surrounding key to genera of Metastigmata (compare keys to species of *Ornithonyssus* and *Psoroptes*).

Although this volume is unlikely to replace or even supplement the more expensive manual by Krantz in laboratory courses in acarology, it will prove a valuable tool for less specialised courses dealing with terrestrial arthropods, and for state and federal workers and other researchers who have occasion to make preliminary sorts of mites to be sent to specialists for determination. □

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A tiger beetle

Animal behaviour

J.P. Hailman

The Ecology and Evolution of Animal Behavior. Second edition. By Robert A. Wallace. Pp.284. (Goodyear: Santa Monica, California, 1979.) Price please. *Animal Behavior: An Evolutionary Approach*. Second edition. By John Alcock. Pp.532. (Sinauer Associates: Sunderland, Massachusetts, 1979.) \$16.00 cloth. *Evolution of Behaviour*. By Jerram L. Brown. Pp.760. (W.W. Norton: New York, 1975.) \$17.95. *Insect Behaviour*. By Robert W. Matthews and Janice R. Matthews. Pp.507. (John Wiley: New York and Chichester, UK, 1978.) \$32.40. *Comparative Animal Behaviour*. By Donald A. Dewsbury. Pp.452. (McGraw-Hill: New York and London, 1978.) \$17.95.

THE closing year of the decade added at least three textbooks on the increasingly popular study of animal behaviour. Robert A. Wallace's *Ecology and Evolution of Animal Behavior* appears in a second edition, with 16 chapters embracing such topics as "classical instinct theory", drive, learning, communication, sex, aggression and social behaviour including altruism and warfare. The frontispiece is clearly a discus (*Symphysodon*), and on page 118 where the figure is repeated we learn that it illustrates "false eyespots near their tail."

The figure might be symptomatic of a second major problem of this book: the common discus species known to aquarium buffs (*S. aequifasciata* and *S. discus*) have no such eyespots, I cannot find such a marking in illustrations of *Symphysodon* species, and an ichthyologically knowledgeable colleague knows of no such species. It would have been useful for the book to identify all species illustrated. Surely the "peregrine falcon" in figure 2.2 is peculiar for the species, and in any case is landing rather than hunting as asserted. Sea horses (figure 2.5) suck up prey somewhat like a vacuum cleaner does, rather than snapping them as asserted. Although some instructors may like the chatty, anecdotal nature of the text, I find it slightly condescending and certainly no paragon for undergraduate writing.

John Alcock's widely used *Animal Behavior: An Evolutionary Approach* appears in a second edition that shows some, but not marked, improvement over the first. This is not a general text on behaviour, but rather is aimed at ecological and evolutionary aspects. There is some material on immediate control of behaviour and its physiological mechanisms, but the author deals only briefly with learning and ontogenetic

development. Indeed, the brief treatment of ontogeny is totally puzzling, as evidenced by such sentences as: "Please note that I am NOT arguing that innate behavior patterns are genetic or more genetic than learned behaviors." When Alcock is in his evolutionary element, however, the text is extremely good, and I believe that a perceptive instructor could utilise it in conjunction with other material.

Because of the similarity with Alcock's book, Jerram L. Brown's *Evolution of Behavior* deserves at least passing mention. The focus is the same, but there is more material on both physiological mechanisms and ontogenetic development of behaviour, making the book more comprehensive, if a few years older. Like Alcock and most other evolutionary behaviourists, Brown finds environmental influences on the development of behaviour slightly annoying, but at least he faces the annoyance reasonably honestly rather than trying to explain it away with semantics.

For entomological ethologists the new *Insect Behavior* by Robert W. and Janice R. Matthews is the first real text of its kind. Although I claim no expertise in insect behaviour, I found the organisation of this book well suited to its subject matter, and those subjects that I know something about to be treated well. Finally, one need mention Donald A. Dewsbury's *Comparative Animal Behavior*, a book (by

a psychologist) with too much elementary zoological material but with a marvellous balance of treatment among the four classes of behavioural determinants — control, ontogeny, perpetuation and phylogeny of behaviour. The text is not, however, orientated substantially toward natural history and field studies, so has not earned plaudits from my students to match my own.

It is difficult to escape the conclusion that a really outstanding text on animal behaviour has yet to be written for a modern audience. Many teachers still rely on one of the three originals, two in revised editions: *Mechanisms of Animal Behavior* by Peter Marler and William J. Hamilton III (Wiley: New York and Chichester, UK, 1966), *An Introduction to Animal Behavior: Ethology's First Century* by Peter H. Klopfer (Prentice-Hall: Englewood Cliffs, New Jersey, 1974) or *Animal Behaviour: A Synthesis of Ethology and Comparative Psychology* by Robert A. Hinde (McGraw-Hill: New York, 1970). Each has its own strengths and weaknesses and all have been well reviewed over the years. A cynic might prefer to follow the dictum of Louis Agassiz: "Learn from nature, not books."

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Introductory chemistry for the health sciences

A.D. Cooper

Chemical Principles for Life. By Lois Forney. Pp.574. (Prentice-Hall: Englewood Cliffs, New Jersey, 1978.) \$14.95. *Elements of General and Biological Chemistry*. Fifth edition. By John Holum. Pp.571. (Wiley: New York, 1979.) \$16.95. *Chemical Principles for the Life Sciences*. Second edition. By Ralph Fessenden and Joan Fessenden. Pp.526. (Allyn and Bacon: Boston, 1979.) \$18.95.

ALL three texts are well written. Forney writes at a higher level than the other authors. Particularly difficult concepts covered by Forney are: thermodynamics, Henderson-Hasselbach equation, coupled reactions, standard reduction potentials and titration curves. Difficult concepts are often covered relatively early in the text.

Holum writes at a lower level than the others. Fessenden and Fessenden write at an intermediate level that can be understood by most students. The authors are patient and approach each topic in a systematic way. Difficult concepts covered by the Fessendens are equilibrium constants and coupled reactions. In general, Forney and the Fessendens define terms more clearly than does Holum.

Of the three texts, Holum's has the most special features. Each chapter has a summary, selected readings and ample questions. Worked out examples are included and answers to in-chapter exercises are at the back of the book. Appendices include mathematical manipulations, inorganic and organic nomenclature, and data on nutrition. At the end of each chapter the Forney text has a programmed review that includes fill-in-the-blank questions with answers in the left-hand margin. Some of the chapters have additional questions and mathematical problems. Worked out examples are included. No appendices are given. The Fessenden text has a set of objectives at the start of each chapter and study questions at the end. Numerous well

worked out examples are included. The appendix on mathematical manipulations is thorough. Answers to odd-numbered study questions are at the back of the book.

The Holum text is well illustrated, with many pictures and coloured plates. However, the illustrations in the Forney and Fessenden texts are more than adequate. The figures of Forney's text are in black with shades of grey. Those of the Fessenden and Holum texts are in shades of blue and grey with section headings in blue. The Holum text contains 22 chapters whereas the Forney and Fessenden texts contain 31 and 30 chapters, respectively. The order of chapters and topics are similar with few exceptions. Nuclear chemistry and radioactivity are treated in Chapter 5 by Forney, in Chapter 10 by the Fessendens and in Chapter 22 by Holum. The treatment of electrolytes, body fluids and acid-base balance is presented in the general chemistry unit by Forney whereas these topics are treated in the biochemistry unit by the Fessendens and Holum. The Fessendens have separate chapters on oxidation-reduction reactions, introducing organic chemistry, introducing biochemistry, molecules in three dimensions (optical isomerism), and a chapter entitled Minerals, Vitamins and Drugs. In contrast, Holum and Forney have only the chapter on introducing organic chemistry.

All three texts give a good treatment of the principles of general chemistry, with Fessenden and Fessenden giving the most careful and straightforward presentation. An integral part of many introductory chemistry courses is mathematical problem solving. The Forney and Holum texts give a modest number of mathematical problems. The Fessenden text gives a much more quantitative treatment than the others. Of the three, the Holum text gives the most abbreviated treatment of organic chemistry. All three cover the various principles of biochemistry in a traditional manner.

Throughout these texts relevant examples and applications from the health science fields are described. Forney gives the most applications, with Holum a close second. The Fessendens' use of relevant examples is more judicious and certainly more than adequate.

Outstanding features of these books include the following. Holum's use of up-to-date relevant examples such as clinical charts and a section on radioimmunoassays. The Fessendens' chapter on molecules in three dimensions is excellent. Forney's discussion of several mechanisms of organic chemistry is commendable because it gives students a rationale for predicting products of reactions.

Weaknesses of these texts include the following. Holum gives very few problems concerning the gas laws and he omits a discussion of competitive versus non-competitive inhibition. His treatment of stereochemistry is very brief. The

Fessendens place the chapter on aromatic hydrocarbons near the end of the unit on organic chemistry. Forney does not include any worked out examples on organic nomenclature and writing structural formulae. Her discussion of stereochemistry in the introductory chapter is much too early.

In summary, the Fessenden text is clear and straightforward. It emphasises chemical principles, contains numerous worked out problems and has a modest number of relevant examples from the health sciences. This text is suitable for a course emphasising a traditional chemistry approach. Of the three, the Holum text has the best blend of worked out problems, relevant examples and chemical principles. It would be suitable for a course requiring a

modest degree of mathematical problem solving, a short treatment of organic chemistry and a lot of relevancy. Forney's text is written at a high level and is demanding of the student(s). It would be suitable for a course that emphasises the chemical principles involved in physiological processes and requires only a modest amount of mathematical problem solving. For our two-semester course designed primarily for students in our Nursing Education Program I would prefer the text by Fessenden and Fessenden. □

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Physical chemistry for biologists

Verne N. Schumaker
E. Reisler

Physical Chemistry and its Biological Applications. By Wallace S. Brey. Pp.589. (Academic: New York, San Francisco and London, 1978.) \$15.95.

SOME, but no detailed knowledge of calculus is required for the study of this text, designed to present the basic principles of physical chemistry with the biologist in mind. The selection of topics is good and the book is easily read. Although the level is elementary, biology majors may find it challenging and it should be considered by instructors who plan to teach at this level.

The first two chapters introduce the kinetic theory of gases, intermolecular forces, Raoult's and Henry's laws, and the colligative properties of solutions. This material expands upon topics usually introduced in first year chemistry courses at about the same level of sophistication. The basic concepts of thermodynamics are presented in the next two chapters. In our opinion these chapters will require much discussion and supplementation by the instructor to impart more than a casual acquaintance with the first and second laws.

A good presentation of the Debye-Huckel theory is given in the chapter on solutions of electrolytes. The elementary algebra of ionisation and titration of weak electrolytes comes next, followed by a

chapter on oxidation and reduction. Biological applications are described mostly in qualitative terms. The electromagnetic and quantum nature of radiation is used to introduce a simple exposition of the photoelectric effect, the Bohr atom, particle spin, and the structure of polyatomic atoms. The chapter on quantum mechanics ends with a presentation of the Schrödinger equation.

The discussion of bonding and molecular spectroscopy is a descriptive overview of these important areas of physical biochemistry. The explanation of covalent bonds is based on the concept of MO, but the LCAO method, although employed in the text, is never explicitly described. Whenever specific examples are used to clarify the introduced concepts (hybridisation of orbitals, electron delocalisation, vibrational spectroscopy) the presentation is clear and helpful. Fluorescence, for all its potential uses in biochemistry, is hardly mentioned. Circular dichroism, though more popular nowadays than ORD, receives less attention than the latter. The qualitative explanation of these techniques is clear, though it lacks somewhat the continuity of presentation.

The chapters on Kinetics of Chemical Reactions and on the Adsorption and Surface Effects are well written. Though brief, these sections focus the attention on areas of great significance. Transport methods and light scattering are described in the chapter on macromolecules. Specific examples or additional illustrations would have helped in giving the students a better feeling for how the actual experiment is conducted, and the data collected and calculated. □

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Introductory biology texts

Edward C. Cox & Robert M. May

Biology. By Karen Arms and Pamela Camp. Pp.970. (Holt, Rinehart and Winston, 1979.) \$17.95. *Life on Earth*. Second edition. By Edward Wilson *et al.* Pp.846. (Sinauer: Sunderland, Massachusetts, 1978.) \$16.95. *Study Guide for Life on Earth*. Second edition. By P.S. Associates and Carl Pike. Pp.281. (Sinauer: Sunderland, Massachusetts, 1978.) \$5.95. *Test File for Life on Earth*. Second edition. By P.S. Associates and Carl Pike. Pp.97. (Sinauer: Sunderland, Massachusetts, 1978.) Free. *Biology*. Third edition. By Helena Curtis. Pp.1043. (Worth: New York, 1979.) \$19.95. *Biology*. Second edition. By James Case. Pp.673. (Macmillan: New York, 1979.) \$17.95. *Biology, The World of Life*. Second edition. By Robert Wallace. Pp.584. (Goodyear: Santa Monica, California, 1979.) \$16.95. *Biology, The World of Life: A Learning/ Study Guide*. Second edition. By Gerald Sanders. Pp.200. (Goodyear: Santa Monica, California, 1978.) \$7.95. *Concepts in Biology*. Second edition. By Eldon Enger, Andrew Gibson, J. Richard Kormelink, Frederick Ross and Rodney Smith. Pp.512. (Brown: Dubuque, Iowa, 1979.) \$13.95. *The World of Biology*. By P. William Davis and Eldra Pearl Solomon. Pp.756. (McGraw-Hill: New York, 1979.) \$16.50. *Biology Lab Manual*. Sixth edition. By A.M. Winchester. Pp.331. (Brown: Dubuque, Iowa, 1979.) \$10.95.

IN the US, there are around 3,000 colleges and universities, ranging from local, 2-year junior colleges to the 100 or so major research universities. The array is, moreover, a multi-dimensional one; entry standards vary widely, and some of the small colleges produce very little research yet do some of the best teaching, while some of the great research universities tend to process, rather than teach, all but a few of their students. This enormous and healthy diversity means that introductory biology courses are taught at many different levels, and college texts are competing in a multifaceted niche space. This is reflected in the character of the introductory biology texts reviewed here.

With differences in tactical detail, all seven books share a common strategy for organising the material. This "little-to-big" strategy begins with the rudiments of chemistry and molecular biology, and proceeds to cellular, organismal and population biology, ending with discussion of human ecology. We now comment briefly on the individual books (taking them in what we judge to be descending order of sophistication), and then air some general opinions.

The book by Arms and Camp is the longest (970 pages of double-column small print) and most consistently scholarly of the seven. The authors explain their aim is

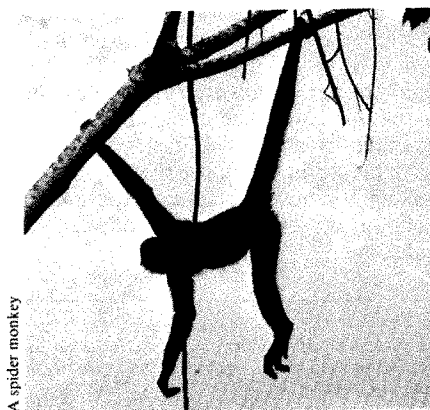
to write a comprehensive text without the usual heavy emphasis on molecular biology, giving a fair share of attention to plants, adaptation and ecology; plants, particularly, tend to get short shrift in introductory texts, as instanced by the other six books here. Arms and Camp's *Biology* has six main sections, beginning with cells, and going on to information coding and transfer, taxonomy and discussion of the major phyla, animal biology (mainly physiology, with two excellent chapters on neurobiology), plant biology, and ecology and evolution. The information is up to date (for example, Chapter 9 treats recent work on recombinant DNA, and ends with two incisive essays on protein sequencing and evolutionary trees), and the many illustrations are cogent. The section on ecology and evolution is comprehensive enough to serve as a text for a basic course specialising in that subject, a claim that could not be made for any of the other books.

Generally, Arms and Camp's *Biology* has an air of authority (surpassed only by the high points in *Life on Earth*). The book is also a well designed learning tool, each chapter beginning with a list of objectives and questions, and ending with a self-quiz, a thoughtful set of "questions for discussion", and references for further reading. One fault is a tendency to excessive codification of organisational structure of the book; this reaches obsessive proportions in the 8-page, double-column table of contents. While one of us (R.M.M.) is greatly addicted to numbering things, it must be admitted that: (AC-1) although pedagogically sound, the practice (AC-2) irritates most students, and (AC-3) is here done in an aesthetically displeasing way. Apart from this minor flaw, and the tendency to excessive compendiousness, the book is an excellent and well balanced text for introductory biology at a good college, covering the field at the right level and in a serious and interesting way.

Life on Earth by Wilson *et al.* is the book we have been using in our own course. The good parts are very good indeed, but (as is hard to avoid with seven authors) the book is patchy both in the topics covered and in the level of presentation. In general, the chapters on cell chemistry, the structure of macromolecules, energetics, population biology and ecology are done well. But parts of the chapters on molecular genetics, classical genetics, and cell chemistry and development are superficial, use metaphors that distract rather than enlighten, or are excessively compressed (sometimes to the point of unintelligibility). The quest for "relevance" is not always fortunate; the two final chapters on alternative futures, "Toward A Stable Ecosystem" and "Defeat by Default", are troughs of insubstantial rhetoric. The book by Curtis has the edge in careful planning, consistent presentation, and crisply appropriate examples from the literature. The strengths of *Life on Earth* are the peaks where people close to the frontier give glimpses that a well prepared student will find new and exciting: the evolution of polypeptide chains; the origins of life on Earth; the molecular details of enzyme action; island biogeography.

Two booklets accompany *Life on Earth*: a *Study Guide* and *Test File*. The *Test File* is simply a compendium of over 1,000 multiple-choice test questions. It is easy to find horrors, but then it almost always is. Chapter by chapter, the *Study Guide* summarises key ideas and terms, gives a quiz for "organizing and testing your knowledge" (which is done well), and gives a sample "self-test". The emphasis is on regurgitating information (as Simon and Garfunkel sing, "All my words come back to me, in shades of mediocrity"), which is not easily avoided in introductory biology texts. Diligent use of these booklets would almost guarantee a student an A in a multiple-choice or short answer examination.

The third edition of *Biology* by Helena Curtis has moved from strength to strength through two substantial revisions. The book has the standard progression from the biology of cells, through the biology of organisms, to the biology of populations. Uniformly, the coverage is thorough in simple and lucid prose, making few assumptions and basing the discussion on the previous chapters. The approach tends to be intuitive, rather than analytical. The book is richly illustrated by spectacular micrographs, colour photographs and excellent graphics. The diagrammatic representation of the Krebs' cycle, for example, uses three colours to lay bare the essentials of the cycle with brilliant clarity. At no point, however, does the book strive for real depth or authority. Beautifully produced and even in tone, it is an extremely good introductory biology text for students who have no extensive science background; but it may lack the spark desirable in a college text for students who



A spider monkey

From *Biology* by Helena Curtis

have a first-rate high school preparation in the natural sciences.

Case's *Biology* is a well written text at the level appropriate for a student with relatively little science background. It begins with chemistry and the beginnings of life, and moves to genetic biology and multicellular life and (briefly) evolution. The second half of the book is devoted to human biology, mainly physiology, with brief excursions into general aspects of population biology and ecology as they bear on human populations and their environmental problems. Various topics are fleshed out nicely by quotations from the original researchers. Among the seven texts, Case deserves praise for making the most substantial attempt to tackle mathematical problems in biology, particularly in quantitative aspects of population growth. The book is well produced (though nothing rivals Curtis). It is broadly at the level of Curtis, differing in giving more attention to human biology and human problems, and less attention to other organisms above the cellular level.

In *Biology, The World of Life* by Wallace, the first 8 pages set much of the tone, juxtaposing scenes from the natural and the man-made world: row houses in San Francisco versus the hills untouched; the northwest face of half-dome in Yosemite versus trash piled in a New York alley; but not, for example, the Taj Mahal versus a naturally oily bog. The basic presentation is thorough, rigorous and well organised in the canonical little-to-big format, but lacking a sense of the excitement of the research frontier. The population biology and ecology sections are at a low level, and are broken up by intrusive moral tales. The illustrations are good, though the graphics are not as good as in the above texts. Much more than any of the other books, Wallace's text conveys a real sense of a characterful individual who really enjoyed writing it. If we must have an author's views on sex or why have children or the sociobiology debate pressed upon us, let it be someone with the sense, sensibility and self-deprecating humour of Wallace. We found his distinctive style to be, in the main, engaging and witty, with occasional lapses into the self-consciously literary or, like, cool ("We've tossed around some heavy concepts fairly loosely here", page 441). The book is designed primarily for students not majoring in biology, which seems right, although if one really warmed to Wallace's idiosyncrasies, one could risk the text on biology majors.

Wallace's book comes with a *Study Guide*, which we think clearly superior to the one accompanying *Life on Earth*. There is some reliance on true/false questions, and on multi-choice, but an attempt is made to get the student thinking as well as mastering detail. The helpful hints (for example, on how to solve genetics problems) seem genuinely helpful.

Concepts in Biology by Enger *et al.* is apparently designed primarily for use in

junior colleges. The chapters on molecular biology and cell structure and chemistry are very simple, but accurate and competently done. Discussion, for example, of photosynthesis, glycolysis and oxidative phosphorylation is to the point and easy to follow. In the absence, however, of a discussion of underlying energetics, the processes lack context and appear arbitrary; a little like memorising Persian. The presentation of organismal biology and ecology is similarly competent, adequately illustrated, but descriptive rather than analytical. The sentences are short, and the general tone seems aimed at an audience with little preparation, not only in science, but in reading or thinking; a random collection of section headings is illustrative ("The environment calls the shots", "Nature throws a curve" (the logistic), "The holey ones — the sponges or porifera", lots of irrelevant cartoons). For the chosen level, the biological coverage is good, but students deserve better prose than this.

The World of Biology by Davis and Solomon is written at a very elementary level. It is given to discussion of the "lifestyle" of organisms, and to "relevance". It also has too many mistakes, which disqualify it as a text. For example, on page 39 it is claimed that radioactive oxygen was used to show that the oxygen in CO_2 appears in glucose, not in free oxygen. Two kinds of experiments were actually done: in one, ^{18}O -labelled water was shown to generate ^{18}O -labelled oxygen by photolysis; in the other, isotopes of carbon, both stable and radioactive, were used to show that carbon from CO_2 appears in glucose. On page 78, the discussion about conformational regulation of enzymes contains a completely misleading example, implying that proteins denature and renature as they go from inactive to active forms within the cell. On page 111, apart from the slangy writing in the first part of the paragraph, it is asserted that 90% of cancer cases are thought to result from environmental factors; this is, to put it mildly, debatable. On page 609, the Scopes trial is claimed to have increased public acceptance of evolution; in fact, its immediate effect was to see references to evolution systematically removed from the next generation of textbooks.

In the table below, we have summarised some of the more mechani-

cally pedagogical features of these books. It must, of course, be remembered that the above list is not an exhaustive list of introductory texts. In particular, the new edition of Keeton will probably be a strong contender in the top category.

Our list of books also included a *Biology Laboratory Manual* by Winchester. This manual sets out 26 different experiments, illustrating many of the chapters in most introductory texts: chemistry; cell movement; seeds; protists; structure of higher plants through animals; the reproductive system; and principles of heredity. For the most part these experiments are purely descriptive, with little emphasis on doing new things or testing unknowns, or the like. We like the chapter on the reproductive system where it is noted (page 296) "Now that we have gotten away from some of the mid-Victorian ideas about anything related to sex", "semen can be collected in a condom during sexual intercourse" for this part of the laboratory. The manual offers no advice, West-Coast-contemporary or otherwise, on who supplies the sample.

Looking over this set of books, as a whole, prompts several thoughts.

The subject matter embraced by biology has grown, and continues to grow, at a prodigious rate. There is an inevitable tendency to gargantuan texts, overwhelming the student with detail and anecdote. One way of coping with this problem is to be selective in the coverage, but as a publication strategy this carries the danger that opinionated reviewers will complain about patchiness, as we have about *Life on Earth*. Another idea, easier said than done, is to focus on a few organising themes and general questions. For example, the question of why are there as many species as there are — a few million rather than billions or thousands — has nagged the seminal thinkers from Darwin to our day. Much of the material on ecology, phylogeny and the fossil record can be organised around this theme, but ultimately our ignorance about the answer to the question makes for a fuzziness that most students find uncomfortable (which is, presumably, why none of the books develops this particular theme). As another example, why do most ecosystems have only three or four trophic levels? A limited number of studies in the 1950s suggested 10% of the energy at one level flows to the

Pedagogic characteristics of the seven texts

Authors	Objectives listed at start of chapter?	Summary at end of chapter?	Glossary of technical terms?	Questions at end of chapter?	References for further reading?
Arms & Camp	Yes	Yes	Combined with index	Yes	Yes
Case	No	No	Combined with index	No	No
Curtis	No	Yes	End of book	Yes	Sometimes
Davis & Solomon	Yes	Yes	Start of chapter	Yes	Yes
Enger <i>et al.</i>	Briefly	Yes	End of both chapter and book	Yes	A few
Wallace	No	Study guide	End of book	Study guide	Yes
Wilson <i>et al.</i>	No	Study guide	End of book	Study guide, Test file	Yes

next, which gave rise to the answer that such attenuation necessarily limits the number of trophic levels to a few. But subsequent work has shown the percentage of energy transfer varies widely among terrestrial and marine communities, and between warm and cold blooded animals, yet the number of trophic levels remains remarkably constant at around three or four (with a tendency to fewer, not more, levels in highly productive environments). Here is a fascinating question, of importance and relevance. Yet only two books (Arms and Camp, and Wilson *et al.*) raise the question as such, and both answer it with the conventional wisdom of the 1950s. All seven books assert the "10% law", and the *Test File for Life on Earth*, indeed, offers a multiple-choice question in which the "correct" answer is that 10% of the energy at one trophic level is transferred to the next, with 5% or 2% being wrong answers; this is fossilised misinformation. In short, the larger questions tend to be lost, and replaced by detail.

The mathematics in these books at most extends to simple algebra and the exponential function. The student is asked to understand the simple mathematics of the Hardy-Weinberg theorem in five of the books (Davis and Solomon mention it, Case does not), but population genetics is

not further developed. It is hard to see how things could be otherwise, given the low and systematically declining (witness the SAT scores) mathematical preparation of students entering college in the US. At the same time, description continues to give way to analytical approaches in biological research, and the gap between what is needed and what we teach in elementary courses continues to widen. There is no easy answer.

With the exception of Arms and Camp, and Curtis, all the books, to one degree or another, preach to their audience about matters to which the authors bring no special qualifications. The classroom setting encourages this self-indulgent evangelism about sex, lifestyles and man's environmental problems, to the point where it constitutes a form of occupational hazard. But there is less excuse for allowing such material to pervade an introductory biology text, much less allowing it to provide the central theme. Examples are Wallace devoting more space to foreplay (2 pages) than to food chains (1 page), Case more or less equating ecology with human ecology, David and Solomon giving as much space to examining yourself for breast cancer as to the history of ideas about evolution, or the discussion of reproduction in *Life on Earth* degenerating into

chatter about Harris' *My Life and Loves*. Relevance is the grail that justifies all this. The pity is that biology in general, and population biology and ecology in particular, offer much scope for the pursuit of important and relevant problems, in a substantial way that lies within the framework of the discipline. Our bad-tempered remarks stem from the belief that the most important goals of college education are to introduce people to sustained and dispassionate analysis, and to teach them to distinguish hard fact from casual opinion; the books do not all serve this cause well.

Finally, notice that the seven books illustrate a form of law of one price. Apart from the paper-bound Enger *et al.*, the books all inhabit the range \$16.50 to \$20.00. The absence of niche segregation by price is understandable, although remarkable in view of the disparities in size, quality of production and quantity of professional effort among the books. □

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Physics for poets

A. M. Sachs

Physics Without Math: A Descriptive Introduction. By Gilbert Shapiro. Pp.351. (Prentice-Hall: Englewood Cliffs, New Jersey, 1979.) \$14.95. *The Ideas of Physics.* Second edition. By Douglas C. Giancoli. Pp.528. (Harcourt Brace Jovanovich: New York, 1978.) \$14.95. *Physical Science: A Dynamic Approach.* By Robert T. Dixon. Pp.402. (Prentice-Hall: Englewood Cliffs, New Jersey, 1979.) \$15.95. *Conceptual Physics . . . A New Introduction to Your Environment.* Third edition. Written and illustrated by Paul G. Hewitt. Pp.660. (Little, Brown and Company: Boston, 1977.) \$15.95. *Instructor's Manual to Accompany: Conceptual Physics . . . A New Introduction to Your Environment.* Third edition. By Paul G. Hewitt. Pp.245. (Little, Brown and Company: Boston, 1977.) Free.

THE four books will be considered here as texts for an elementary and usually terminal physical science course for non-science college students, who often refer to it as physics for poets. (Giancoli's and Hewitt's books are also intended, as

Hewitt states, "as a springboard to a greater involvement in physics".) Although this reviewer is presently teaching such a course, he has neither used these texts nor had the opportunity of observing the reaction of students who have read them. On a larger scale, it is unlikely that any text will gain widespread acceptance in this area, since each teacher seems to have his own ideas in particular as to how a physics course for liberal arts students should be designed.

These four books explicitly avoid mathematics and either under-emphasise or omit entirely quantitative problems. In *Physics Without Math: A Descriptive Introduction*, Shapiro states that "it is possible to learn about the scope and power and, yes, beauty of a scientific explanation of nature without solving equations". It is possible, perhaps, but the question could be raised as to whether the psychological gain for some students with "built-in bias against science" is worth the verbal complication for others in his statement, for example, of Kepler's third law: "The ratio (distance times distance times distance) divided by (period times period) is the same for all planets." Giancoli in *The Ideas of Physics*, does use equations in the text and gives some derivations in footnotes, and — presumably to broaden the prospective market — indicates that an "Optional Mathematical Supplement" is available.

All the books cover broad areas of physics, with a varying proliferation of topics. In common with most introductory physics texts, there is little continuity between sections. The second half of Dixon's book *Physical Science: A Dynamic Approach*, is devoted to a survey of chemistry, geology and astronomy, so the 200-page coverage of most areas of physics in the first half is probably too superficial for a course devoted entirely to physics. Giancoli's book and Hewitt's *Conceptual Physics . . . A New Introduction to your Environment*, follow the conventional order and exhaustive list of topics of traditional texts, including fluids, sound and geometrical optics, as well as introducing relativity. Shapiro does not hesitate to omit some topics entirely and to skip over the details of others, while concentrating on introducing enough physics to permit discussion of major areas such as energy and the 'frontiers of science'.

All the authors stress to some extent the historical development of science, as, for example, in the familiar treatment leading from the description of planetary motion to the formulation of newtonian mechanics. Shapiro and Giancoli are a little more consistent in maintaining a historical perspective, but most of the references after the eighteenth century are primarily to names and dates.

Shapiro is concerned with the basic

concepts of physics; a limited number of specific phenomena are described, primarily as examples of these concepts. Hewitt is more directly concerned with 'how things work' and describes, somewhat superficially, many devices such as an aneroid barometer, microelectronic circuits, a ruby laser and a fluorescent light. Giancoli takes more of a traditional approach, stressing important concepts but with a tendency toward describing a wider variety of phenomena.

The four books vary considerably in their scientific sophistication, the liveliness of their presentation and the visual appeal of their format. Shapiro's book displays a deep understanding of physics, with a number of unconventional approaches such as introducing mechanics through the concept of energy and defining force, pressure, surface tension and torque as ratios of "energy transferred" to distance, volume decrease, surface increase and angle, respectively. A serious student can gain significant insight from the book, especially if guided and reinforced by an

accompanying course; less intensive reading of the book, however, is likely to lead to little more than a familiarity with the vocabulary. Unfortunately, the formal tone of exposition and the lack of liveliness in the illustrations and format make it unlikely that most non-science students will be spontaneously attracted to the book.

The visual features of Giancoli's book, on the other hand, are unusually attractive. There is an abundance of lively, and often humorous, two-colour illustrations, imaginatively designed to attract attention as well as to give insight into physical principles. The pedagogy and organisation of the text are more conventional, except perhaps for a series of "experiment-projects" to be performed independently by the students. The book attempts to cover a very large number of topics by presenting and discussing results usually without derivation. For a 'physics for poets' course, it would seem preferable to treat a limited number of topics in greater depth.

Hewitt's book has some of the same visual advantages as Giancoli's. In its 650 pages, however, it tends to be even more encyclopaedic, while consciously being even less quantitative. I fear that its admirable (but not subtle) attempt to relate to students with scientifically disadvantaged backgrounds will unfortunately backfire for most liberal arts students. Some of its chapters start with full-page photographs of children with comic-strip-like 'balloons' containing expressions such as "man-o-man-you should've seen the wild spills I used to take before I got into wide wheel bases", before the chapter on rotational motion. The imagination and humour in the book's many small illustrations seem appropriate for all students; the occasional use of a less mature approach and vocabulary may give many students the impression that the author is talking down to them. □

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Introductory meteorology

Ira W. Geer

Fundamentals of Meteorology. By Louis J. Battan. Pp.321. (Prentice-Hall: Englewood Cliffs, New Jersey, 1979.) \$14.95. *The Atmosphere: An Introduction to Meteorology.* By Lutgens and E. Tarbuck. Pp.413. (Prentice-Hall: Englewood Cliffs, New Jersey, 1979.) \$14.95. *A Basic Meteorology Exercise Manual.* Revised edition. By Chelius and Frenz. (Kendall/Hunt: Dubuque, Iowa, 1978.) \$7.95.

THE three publications reviewed here are intended for introductory college-level courses in meteorology for students not majoring in the atmospheric sciences.

By far the best of those examined is *Fundamentals of Meteorology* by Battan. Clearly written and highly readable, it treats topics found in most introductory weather texts and includes chapters on atmospheric optics and acoustics, and weather applications. Reflecting the author's background and interests, frequent referral is made to basic physical principles and relationships which in turn provide the foundation on which subsequent descriptive information is based. Various aspects of air pollution are presented in ways which will no doubt enhance student interest in a number of topics discussed. The book is relatively free from typographical errors or mistatements. However, one is the mention of South Atlantic hurricanes, when hurricanes in the southern North Atlantic

Ocean was obviously intended (page 183). This book is recommended without reservation for use as a course text, a handy teacher reference, or just plain good reading.

The Atmosphere by Lutgens and Tarbuck was an attempt to write a non-technical and non-mathematical treatment of weather and climate. It seems to be geared to the reading and interest levels of the poorly prepared student. Despite the good intentions of the authors, the book fails miserably. It is lacking in scientific accuracy and quality of writing. The concept of (air) pressure is frequently equated to force (pages 10,121,122 and 124). This leads one to conclude that the authors fail to demonstrate an understanding of basic physics, let alone its applications to the atmospheric environment. Statements about "the infinite universe" (page 1) and that "air condenses" (page 233) illustrate the multitude of errors which at best can be called sloppy writing. Simply stated, the book cannot be recommended for any purpose.

A Basic Meteorology Exercise Manual by Chelius and Frenz is "for students of basic meteorology to be used as a supplement to a lecture course or used in an independent laboratory course". Twelve exercises are presented on topics entitled Temperature, Moisture, Winds and Pressure, Clouds and the Precipitation Process, Fronts and Extra-Tropical Cyclones, Upper Air Flow, The Thunderstorm, Climatology, Air Pollution, Weather Radar, Weather Forecasting I and Weather Forecasting II. The bibliography contains a brief list of books and other sources of information.

The manual's sections touching on

satellite imagery, air pollution, weather radar, the relating of surface to upper air weather features, and weather forecasting are somewhat unique as they are not typically treated in the few other basic manuals available commercially. They add a great deal to a publication which is weak in terms of its layout and the quality of its narrative portions. Statements such as "the condensation of water vapour produces precipitation" (page 15) and the description of saturated air as being "like a sponge that can hold no more water" (page 19) appear too often. Unfortunately, student analysis of data from actual atmospheric observations is not often called for. In its present condition the manual cannot be recommended for course adoption. Teachers might find a copy useful for ideas they might want to develop on their own. With considerable revision, the manual could become suitable for general use. □

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Chemistry for the non-science student

A.P. Zipp

Inside Chemistry. By Charles Compton. Pp.569. (McGraw-Hill: New York and London, 1979.) \$16.95.

IN the words of the author, this text was written to "meet the special needs and interests of nonscience students" while having enough flexibility to "provide the basis for courses with different emphases, levels and lengths". In my opinion he has been reasonably successful in achieving his goals, although the book has limitations.

On the positive side, the selection of topics seems sufficiently broad to satisfy most instructors, although several lack depth (see below). The text is divided into eight chapters (297 pages) of 'core' material and seven chapters (200 pages) of

'applied' topics, with each of the latter being independent of the others so that they may be studied in any order or combination. In addition, most chapters contain optional sections and the bulk of the quantitative material has been gathered into supplements at the end of the text. These features should allow each instructor to adapt the book to his own course.

The book is written at an appropriate level and the examples used should be of interest to the intended audience. The learning process should be enhanced by the self-study questions (with answers), the practice exercises (without answers), and the key word lists which follow each chapter as well as the excellent glossary at the end of the text. The data and statistics are as current as could be expected, a point which is particularly important for environmental courses, and each chapter has a list of relevant references (some as recent as 1977).

On the negative side, the book is occasionally redundant, although this occurs mostly in the later chapters which are intended to be independent of one

another (for example, photosynthesis is discussed in three different places and production of carbon monoxide in two). More seriously, some discussions lack sufficient depth to be understood readily. Specific examples include Dalton's atomic theory, the greenhouse effect, base pairing in nucleic acids, oral contraceptives, and amino acid balance in foods. Although this can be remedied in lecture, it may be distressing to students who consider a text to be the final authority, and instructors who feel similarly may wish to select a book designed for a course with a particular emphasis (for example, environmental or biochemical).

In summary, this is a potentially useful text with enough strong points to justify its consideration by instructors of non-science major courses but should be recognised as one which will require supplementary work by the teacher in many areas. □

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Aquatic insects

A.A. Grigarick

An Introduction to the Aquatic Insects of North America. Edited by R.W. Merritt and K.W. Currins. Pp.441. (Kendall/Hunt: Dubuque, Iowa, 1978.) \$21.95.

AQUATIC insects have been receiving a great amount of attention from many scientific disciplines for the past 20 years. During this period a considerable number of taxonomic changes, new information on distribution, and ecological studies on aquatic insects have occurred. Merritt and Cummins have gathered the contributions of an impressive group (21) of scientists to incorporate much of this information into *An Introduction to the Aquatic Insects of North America*. The book was directed at both students and professionals of the many fields that deal with the aquatic habitat and the authors do not assume the reader to have an entomological background.

The first chapter explains the make-up of the book, indicates its limitations and presents an annotated list of general taxonomic references dealing with aquatic insects. Enough general morphology is given in Chapter 2 to lay the groundwork for use of the keys. Chapter 3 is devoted to collecting, sampling and rearing methods which are tabulated by habitat and community. This chapter includes an amazing amount of information in a well organised and concise form and it is well documented with source material and illustrations.

A short chapter on ecology and distribution sets the format for the handling of this information in later chapters at the generic level in each order. The tabular presentation of information includes habitat classification, locomotive behaviour, trophic relations (based on feeding mechanism) and distribution. By necessity, this information is often based on generalisations of limited numbers of species but when it is used in this light and with the supporting references it is of considerable value. In table 4C some of the examples of orders used to demonstrate trophic classification and columns of the classification are either out of alignment or wrongly set up at the printers. The reader should obtain this information directly from the trophic relationships presented at the end of the chapter for each order.

Phylogenetic relationships and evolutionary adaptations of aquatic insects are briefly reviewed in Chapter 5. Chapter 6 takes up the entomological basics of classification and metamorphosis and a key to immatures and adults is given for the orders with aquatic or semi-aquatic insects. These orders are taken up in a phylogenetic sequence in the remaining chapters. A general introduction, pertinent external morphology and keys to the family for immatures and adults are given for each order. Key characters are usually well illustrated and additional taxonomic references follow the keys. Each chapter concludes with a tabular summary of ecological and general distributional information at the generic level.

The keys to Megaloptera, Neuroptera and Lepidoptera (in part) allow identification to genus. About one-third of

the book deals with the aquatic Diptera and includes separate chapters on the Tipulidae, Culicidae, Simuliidae and Chironomidae. The expanded treatment of these families includes keys to immatures and adults to genus for the first three but is limited primarily to tribe for the chironomids. The limitation of the taxonomic treatment to the family level for most orders was disappointing to many researchers and instructors of courses in aquatic entomology. With due respect to the large size of this group of organisms and incomplete knowledge of some taxa (for example, Diptera), the taxonomic information for identification to the generic level has been developed for most families. Identification to genus would certainly be desirable in this book and would appear to have been possible if a comparable amount of effort had been made in this direction as was devoted to summarising ecological information to genus.

As the title states, the region of this book's coverage is North America. However, the great diversity of aquatic insects in North America (over 10,000 species), the degree of ecological coverage, excellent illustrations and extensive source material (1,712 references) make this book very useful to aquatic biologists throughout the world. It is particularly welcomed by instructors of the subject. □

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13 March 1980

Dioxin and 2,4,5-T: what are the risks?

A court case due to take place on Monday will decide for, or against, the use of the herbicide 2,4,5-T in the United States. Two old adversaries, the Dow Chemical Company and the Environmental Protection Agency, will argue the case over the legality and validity of the partial ban on 2,4,5-T currently in operation in the US. Dow Chemical, the largest manufacturer of 2,4,5-T in the world, will challenge the ban imposed by the EPA in March of last year; it is Dow's view that 2,4,5-T poses no risk to users.

There are those in the EPA who now believe that Dow will win the case. The Agency's own study linking 2,4,5-T spraying with an increase in spontaneous abortions in the state of Oregon — information on which the decision to ban the herbicide was based — apparently has serious flaws. The study has been severely criticised on methodological grounds by many independent scientists, and at least three reports have been produced opposing the EPA's findings. They argue that the areas of land chosen in the original EPA report were not carefully matched and therefore could not be regarded as truly representative of a 2,4,5-T sprayed area and control area. In addition, differences in hospital admissions for miscarriages vary among the regions chosen for study, and these practices, critics charge, were not given due consideration in the original study.

Such criticisms may be justified, and a reappraisal of the Oregon study, taking these additional factors into account would probably remove the association between miscarriages and 2,4,5-T spraying.

Evidence that the EPA is indeed moving away from a reliance on the Oregon study is provided in the Federal Register of 13 December, 1979. The Agency now appears to be basing its decision to call for a total ban on 2,4,5-T on the fact that the 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) contaminant present in the herbicide is an established animal carcinogen. Many oncologists now consider that there is no safe threshold dose for a carcinogen, and the EPA considers, therefore, that to expose the public to such a chemical by permitting usage of 2,4,5-T poses an unacceptable risk.

There are many in the EPA who believe, however, that in spite of the presence of dioxin in 2,4,5-T there is still no hard evidence of a health risk from the herbicide. A recent request from Dow Chemical under the Freedom of Information Act for all of the EPA's documentation on 2,4,5-T could well provide confirmation of the lack of this evidence. If Dow's request is

granted, the EPA's case for seeking a permanent ban on the herbicide could well be undermined.

Evidence elsewhere against 2,4,5-T is not very strong either. The best study to date was completed in Sweden and produced evidence that among those exposed to 2,4,5-T and chlorinated phenols in the course of their work in the lumber industry, there was a six-fold increase in soft tissue sarcomas. But even in this study confounding factors may not have been taken fully into account and the Swedish government is reassessing it.

It is therefore quite clear that more good and reliable data need to be collected for a proper assessment of the hazards of exposure to 2,4,5-T and its contaminant, dioxin. The best place to seek such data is in the medical records of those who have been or will be involved in the manufacture of 2,4,5-T, since they are most likely to be at risk. In particular that is so because dioxin, which poses the greatest potential hazard, is generally present in larger concentrations during the manufacture of 2,4,5-T than during its use. And to conclude that 2,4,5-T is safe to use without reviewing the full evidence on industrial exposure, as did the UK Ministry of Agriculture, Fisheries and Food last March, is distinctly unsound.

At present the available industrial data are limited and concentrate on exposure after accidents. There is some evidence for a clustering of gastro-intestinal cancers among BASF workers who were exposed to dioxin in an accident in West Germany in 1953, and for an increased rate of heart attacks after an industrial accident in Amsterdam at the Philips Duphar plant in 1963. Monsanto have studied the after-effects of a 1949 accident, and concluded that among affected workers there was a normal incidence of cancer and a lower than normal incidence of heart attacks (*Nature* 14 February, page 613).

More industrial data are needed and must already be available both from the UK firm of Coalite and Chemical Products Ltd., whose reluctance to reveal its data we reported last week, and elsewhere. It is therefore essential that Coalite should reveal the study it has already done and conduct further studies using proper controls and including all those exposed to dioxin whilst in its employment. And when it has done so it must pass the data on to the two bodies which are accumulating a world data-base on 2,4,5-T: the US National Institute of Occupational Safety and Health and the International Agency for Research on Cancer. Only when good studies are complete and openly available can the air be cleared for or against 2,4,5-T. □

United States

Pressure mounts to resume chemical weapons production

REPORTS of the use of poison gas by Soviet troops in Afghanistan are being used to help generate support for allowing the US military to resume the production of chemical weapons banned since the late 1960s.

Supporters of such a move argue that there is considerable evidence that the Soviet Union already possesses a chemical warfare capability considerably superior to that of the US — and that the reports from Afghanistan indicates its willingness to use it.

Critics, however, argue that many widely-quoted estimates of the Soviet stockpile of chemical weapons are greatly exaggerated, and point out that there is as yet no confirmed evidence that Soviet troops have used anything more than riot control agents against Afghan forces.

The US army has been pushing for several years to proceed with production facilities for a new generation of so-called binary weapons, in which two non-lethal gases combine on firing or impact to form a deadly mixture. Such weapons have been under research since the mid-1960s.

Congress rejected this proposal in both 1974 and 1975. More recently, plans for \$111 million production facility was cut by the White House from the Defense Department proposed budget for the fiscal year 1981, currently before Congress.

But pressure is building for the proposal to be reinstated. And at the same time, the Pentagon is increasing the scope of the plans that it would like to put into effect. In January, Defense Secretary Harold Brown announced that, whereas previously it had been intended only to produce projectiles containing the two gases, plans are now being drawn up for producing binary bombs and warheads as well.

Last week the possibility of moving to production came even closer when William Perry, Defense Under Secretary for research and engineering, signed a memorandum instructing the department to complete designs for such a facility and draw up a construction schedule.

How sympathetic Congress is likely to be towards a request for funds for this purpose remains unclear. But a considerable number of articles have appeared in the US press in recent weeks focusing both on the claims about the use of poison gas in Afghanistan (which even the Central Intelligence Agency, after careful study, is said to have been unable to confirm), and more broadly on the apparent inferiority of the US's chemical weapons capability.

An article in *Time*, for example, states that "without a credible chemical counter-punch, it becomes more likely that the US

would have to resort to tactical nuclear weapons as a response to a Soviet attack".

Dr Matthew Meselson, professor of biology at Harvard University and a long-time opponent of chemical and biological warfare, claims that many of the current estimates — such as one that the USSR has a stockpile of 350,000 tons of chemical weapons, compared to only 42,000 tons in the US — are unrealistic and based on faulty speculation, even according to some Defense Department calculations.

"I am concerned that making policy by excitability is likely to lock us into a series of policies which are unwise from a defence point of view, since I do not think that

chemical weapons are that useful in combat situations", Dr Meselson said last week.

The pressure to reintroduce chemical weapons production has coincided with the latest round of chemical disarmament talks between the US and the USSR in Geneva. At the end of the last round in August 1979, US officials expressed optimism that real progress towards an agreement banning such weapons was beginning to be made.

Some now argue that, if the US steps up its production, this will provide added pressure for an agreement. Others, however, argue that it could create a new stumbling block to the negotiation. □

Princeton and Saudi universities sign life sciences agreement

PRINCETON University and the University of Riyadh in Saudi Arabia have signed an agreement under which each university will assist the other to build up a centre for training and research in the life sciences.

The Saudi Arabian university will donate \$5 million to Princeton, a substantial proportion of which will be used to modernise facilities in the university's Moffett Biological Laboratories, and the rest of which is likely to be used to support a number of new faculty posts in the biology department.

In return, Princeton has agreed to help the University of Riyadh to build up graduate and postdoctoral training and research facilities in the life sciences. This will be done both by advising on course curricula and the selection and training of personnel, and assisting with any necessary technical advice.

The agreement was signed in Riyadh last week by Princeton president Dr William G Bowen and the president of the University

of Riyadh, Dr Mansour Al Turki. It follows five years of negotiation between the two universities leading to what is believed to be the biggest single grant from an oil-producing state to an American university.

Dr Edward Cox, Chairman of Princeton's Biology Department, told *Nature* last week that the money would be used to provide additional support for fields of research, such as developmental biology, in which the university already has a strong research tradition.

Faculty members are currently debating the best way to use the new funds to support research salaries. "One view is that you could use the money for assistant professors, because it is the young people who are most in need of jobs", Dr Cox said. It might be possible to offer a number of five or six year fellowships.

In return for the financing, the biology department will assist the University of Riyadh to set up an international life sciences centre for postgraduate students and postdoctoral fellows, most of whom now have to travel abroad to receive their research training.

Several previous attempts to establish cooperative agreements between US universities and Middle East countries have stumbled over the refusal of the latter to accept non-discrimination clauses — leading to the fear that women, Jews and other minority groups might be excluded from participation.

The two universities have circumvented this problem by agreeing that scholars, technical experts and students will be proposed and received for exchanges "on the criterion of merit" rather than according to any other consideration.

David Dickson



"No!! We won't be needing any cheer leaders!"

Research agencies prepare to tighten budget belts

MAJOR cuts in the US science budget which could virtually eliminate any real growth in research funding over the next eighteen months are expected to result from President Carter's moves to reduce federal spending in an effort to lower the rate of inflation.

No cuts have yet been officially decided. But the President is expected to make public within the near future how he intends to move towards a balanced budget by eliminating about \$4 billion of federal expenditure in the current fiscal year by cutting back on commitments already made, and \$15 billion in the 1981 fiscal year which begins on 1 October by reducing the budget request to Congress.

Virtually the only area expected to emerge unscathed is the defence budget. And since the science budget is an area of discretionary expenditure — unlike social security, for example, where expenditure is mandated by law — it can expect to shoulder a fair part of the burden.

Research agency heads were asked last week by the Office of Management and Budget (OMB) how they would meet given targets for reducing expenditure. Among the cuts under discussion are thought to be:

- National Institutes of Health: OMB has asked for cuts of \$170 million in the rest of the 1980 budget, and \$340 million (about 10% of the total) in the 1981 budget as part of a \$700 million reduction for the Department of Health, Education and Welfare.

One immediate casualty could be the administration's attempt to maintain the number of new competitive research grants at about 5000. This may be reduced to about 3,500, considerably raising the necessary quality of applications likely to be funded. There would also be a general cutback across the whole range of NIH's research activities, possibly resulting in the loss of some scientists' jobs.

- National Aeronautics and Space Administration: OMB is said to have asked NASA to prepare for cuts of \$760 million, a sum equivalent to 14% of the civilian space budget. This would affect almost all of the NASA's programmes, in particular the launch of the space shuttle.

Various planetary missions, such as the Galileo launch to Jupiter, and the solar-polar mission to be carried out jointly with the European Space Agency, would have to be put back, and perhaps even eliminated. The gamma ray observatory, recommended as a new start for 1981, may also be affected.

- National Science Foundation: cuts in the order of \$90 million to \$100 million — about 9% of the proposed 1981 budget — are under discussion with OMB. The budget office has suggested that where possible basic research expenditures should be protected. The main impact is therefore likely to fall on areas such as applied

science and science education.

- Department of Energy: several large-scale energy research projects have to be abandoned or cut back. Among those being closely studied last week in solar energy, for example, were the "power tower" under construction at Barstow in California, and the Ocean Thermal Test Facility (OTEC), now nearing completion off Hawaii.

- National Bureau of Standards: OMB has asked for cuts of about \$7 million, and bureau officials have indicated that at the top of their list is likely to be a new \$5 million initiative to enhance basic research in the agency, a package of eight proposals included in the original 1981 budget request with the specific endorsement of

the Office of Science and Technology Policy.

Administration officials stressed last week that most of the potential cuts were still under negotiation. But President Carter warned state and city officials visiting the White House to expect some unpleasant surprises in the near future — and discussions were taking place last weekend to put together the final package.

If the cuts are made as expected, then they are unlikely to be strongly resisted by Congress, particularly in an election year when many voters will be looking for signs of financial stringency, and to oppose the cuts would be to oppose the President's anti-inflation strategy.

David Dickson

Environment



Lowland tropical rainforest destroyed by road building in the Amazon Basin.

World Conservation Strategy sets priorities for global resources

THE latest international environmental initiative was launched simultaneously last week in 32 countries, amid hopes that the time is ripe to stop squandering the Earth's natural resources. In producing its *World Conservation Strategy**, the International Union for the Conservation of Nature (under the auspices of the United Nations Environment Programme and the World Wildlife Fund) has brought together a wide range of expertise to set out priorities for action to conserve and replenish global resources, from microbial cultures to tropical forests. The heaviest burden of such action would inevitably fall on developing nations, whose will may be impeccable, but whose means may be totally inadequate as the sponsors are bound to admit when they urge policy

makers to take up the strategy.

The major theme of the strategy — that conservation and development must go hand-in-hand has been gathering strength since the UN Conference on the Human Environment in June, 1972. Now the call is for governments and international agencies to base their development programmes on sustainable use of species and ecosystems, which means spending the interest while keeping the capital. The authors of the strategy cite familiar worldwide evidence that much of that capital is being dissipated. Many losses are due to overfishing in the oceans, overgrazing on the land, clearing of forests and destruction of wild animals and plants, often because people are too poor to replenish their resources, even if they take a long term view.

The way to reverse the trend is not by piece meal attempts to save resources once

*Available from the World Wildlife Fund, 29 Creville Street, London E.C.1, £3.60; also available as a book, *How to save the World*, Kogan Page, £2.95 paperback, £5.95 hardback.

they are threatened with destruction. According to the strategy, governments should consider the needs of conservation at all stages of development. As an example, the authors recommend that forestry should not be directed only towards increasing the yield of products and the provision of services such as recreation and education. Forest management must ensure that yields are sustainable, and genetic diversity is preserved. The preservation of genetic diversity in general is an immediate task for governments in order to prevent the extinction of further species of wildlife, crop plants and livestock, any of which could be needed in the future (for example, in agricultural breeding programmes or the production of drugs). The recommended approach is to preserve ecosystems intact within reserves, augmented by collections both of whole organisms, as in zoos and botanic gardens, and of genetic material such as seed and semen.

The preservation of genetic resources is also a priority for action by international agencies, which are called upon to provide a wide range of assistance such as that provided for crop plants by the International Board for Plant Genetic Resources. According to the strategy, financial assistance should also be sought from commercial enterprises that benefit from the exploitation of living resources.

Another international priority is action to halt the depletion of shared resources in oceans and river basins. The scale of losses also makes tropical forests and drylands a priority for international action. In the latter case, low rainfall, high rates of evaporation and inefficient use are encouraging the rapid spread of deserts. The strategy calls for the implementation of the wide ranging plan of action formulated at the UN Conference on Desertification in September, 1977.

The hazards that beset a scheme for environmental action, were well expressed by Mr Michael Heseltine, UK Secretary of State for the Environment, when he said at the launching ceremony in London:

"The basis of my approach — and indeed of the present government — rests on conservation, good husbandry and wise use of resources. But it would be less than frank of me to assert that all decisions of government flow naturally and easily from that premise".

It is, he pointed out, almost always a matter of balancing a developmental need, often with a clear economic advantage, against a potential conservation loss. Mr Heseltine injected a further note of caution when Mr David Attenborough, naturalist and broadcaster, insisted that the people at large should compel governments everywhere to recognise the strategy, which must become a political issue. Otherwise, Attenborough said, it was doomed to be "just another abandoned lofty plan". He has set his fellow citizens a difficult task.

Mary Lindley

United Kingdom

Fast reactor fuel cycle to begin

THE United Kingdom Atomic Energy Authority has been given approval to make regular shipments of plutonium nitrate in water solution from Dounreay on the north coast of Scotland to Windscale in Cumbria, north-west England, as part of an experimental fast reactor fuel cycle. The nitrate will be the result of reprocessing of fuel elements from the Dounreay prototype fast reactor (PFR). For economic reasons the Authority plans to refabricate new fuel, perhaps by the 'sol gel' process, at Windscale rather than at Dounreay. Shipments will begin later this year, at the rate of one every two to four weeks.

Behind the decision may lie uncertainty about the future siting of the commercial demonstration fast reactor (CDFR) that the Authority would like to build. The government has promised a public enquiry on the fast breeder before any commitment is made to the CDFR, and only if it were to be built at Dounreay would it be economic to build a fuel fabrication plant there.

The shipments ultimately will be on a large scale, involving some 800 kg of

plutonium a year. Each load, carried in a specially designed 250 litre flask in a 27m³ containment vessel, may amount to a critical mass of the metal — so the flask has had to be designed to minimise risk of criticality.

Another design problem has been the continuous radiolysis of water by alpha emission from the plutonium. This results in a build up of hydrogen and oxygen gas, in explosive proportions. An inert gas atmosphere is introduced to dilute the gas, and so reduce the risk of explosion.

"Nevertheless", says a Department of Energy note on the shipments, "assessments have been made of the possible consequences of gas ignition within the package".

The package is unlikely to be damaged in a transport accident sufficiently to breach the inner vessel, says the note. "However, if the vessel was ruptured, and in the further unlikely event that a source of ignition was present, and that the mixture of hydrogen and oxygen was in the explosive range, any resultant explosion internal or external to the containment vessel but inside the outer package would generate insufficient pressure to rupture the external structure."

The inner vessel is designed to withstand the pressure that, it is calculated, will build up in the vessel up to 250 days; then it is unlikely to rupture. The danger here is of a shipwreck, followed by a delay in recovery. The Authority has performed recovery tests on a dummy container hidden in a wreck off West Scotland, which "was found and retrieved in hours". However, tests have not been made on the time for which a damaged inner container (after a collision, say) would withstand pressure build-up.

In an impact test, however, there was cracking of the outer vessel and fracturing of the radiation shield. According to a report prepared by the Nuclear Installation Inspectorate, published last week "it can be argued that would give enhanced access to heat flux" in the case of an impact followed by fire. Although, said an Authority spokesman, "that was one we were a bit bothered about", the NII concluded that thermal tests that had been conducted (on an undamaged third-scale model) were "an adequate demonstration" of safety.

The greatest dangers may occur during the loading and unloading of the inner vessel to and from its impact-resistant container, where the Authority has assumed that the vessel would not survive a two-foot drop. But, says the Department of Energy, the Health and Safety Executive are satisfied, as a result of tests, that the vessel would survive such an impact.

Robert Walgate

Poland



Bartosiewicz: power cooperation

Energy agreement

MR Zbigniew Bartosiewicz, Polish Minister of Power and Atomic Energy has signed a cooperation agreement with Sir Francis Tombs, Chairman of The UK Electricity Council. The agreement covers the exchange of information and possible joint research on the more efficient use of conventional energy sources for power generation.

The Minister's five-day visit to the UK comes at a time when Poland's industrial expansion programme has outstripped generating capacity.

During his visit, Bartosiewicz attended a seminar organised by the Department of Industry, at which one of the major topics discussed was "down time" — the unscheduled stoppage of generating sets for maintenance work. UK participants in the seminar later reported that they felt the optimisation of maintenance logistics could well be a fruitful theme for cooperation.

Vera Rich

High-energy physics

Truce and a German director for CERN

PROFESSOR Herwig Schopper, presently director of the Deutsche Elektronen Synchrotron Laboratory (DESY) near Hamburg, has been designated director-general of the European sub-nuclear physics laboratory, CERN, Geneva. He will succeed the present joint directors-general, Leon Van Hove and John Adams, on 1 January 1981.

The appointment has not been without its drama. The Italian delegation, one of the 12 national representations on CERN's governing Council, had been refusing to accede to Schopper's appointment until possible conflict between future CERN and DESY programmes is resolved. But after an extraordinary statement from CERN and another from the Italian Ministry of Science, the air was cleared last week.

The CERN statement describes the meeting of the Committee of Council on 29 February. The programme for the construction of LEP, the large electron-positron collider which is CERN's next major project, was raised.

The statement said:

"The delegates at the Committee of Council agreed with the Italian delegation that LEP must have the top priority among the European accelerator projects in elementary particle physics, and that it should be built as soon as possible. The delegates also agreed, following an Italian proposal, that a study group be formed to discuss certain legal and budgetary problems and the relation of LEP to national programmes . . .

"The Committee of Council entrusted Professor Schopper with the mandate to present Council, at its session in June 1980, with his proposals concerning the top management structure and the directing staff of CERN . . ."

The 'legal and budgetary problems' refer to the question of whether LEP could be built within CERN's present budget, without declaring LEP as a project requiring special funding. The 'national programmes' referred to mean largely HERA, a proposal at DESY to build an electron-proton collider; its inclusion in the CERN text is something of a coup for Italy, which has been worried that HERA would compete with LEP.

In fact on many issues Germany and Italy have come extremely close, both wanting to push LEP along as fast as possible, at the lowest practical budget.

"I believe we can build the first version of LEP to give beam at the end of 1986", Schopper said on Monday. He will now participate actively in constructing the final proposal for LEP, which will be made to Council in June.

Whether LEP will be a CERN supplementary programme, in which the

participation of individual countries is optional, or part of the basic programme, in which it is not, is a crucial question, Schopper believes. "We must try to activate all sources inside CERN", he said, "and that requires flexibility to transfer people from one project to another". But if LEP is merely a supplementary programme, flexibility will be difficult to achieve.

Schopper thinks that LEP can be built "within a few per cent" of the current CERN budget of SF 590 million a year.

Italy reacted to CERN's announcement with its own statement. The Science Minister, Vito Scalia, said last week:

"... we are finally approaching the solution to defining the programmes of CERN. This has been the aim of the Italian government since November. . .

"For the first time in 25 years, CERN had found itself in difficulties in defining its programme against serious competition from other national laboratories. By the end of last year, the pressures we had been applying to reach a rapid clarification of these problems had had no appreciable result. Council opposed our proposal to

discuss the future of CERN, and the Italian delegation decided to underline its dissent. [The delegation walked out of the December Council meeting, an event unprecedented in CERN history. The meeting was to have appointed Schopper.]

"But last week the Committee of Council demonstrated unequivocally that our action, which was intended to give priority to the discussion of CERN programmes over and above the appointment of a new director-general, was fully justified . . .

"All delegates at the meeting of 29 February have given LEP an absolute priority so the new accelerator will probably be built in five years. This decision allows the laboratory to keep its position of pre-eminence and confirms the validity of the 'active Europeanism' that the Italian government has done its best to promote in international research.

"In view of this development it is clear that the recent solution of the CERN case represents a success for my country . . . It clarifies also why the Italian delegation is now disposed to vote for Professor Schopper . . ."

Robert Walgate

Interferon

US company increases production ten-fold

THE US drug company G D Searle & Co announced last week that it plans to increase its fibroblast interferon production "six to tenfold" by the middle of 1981. Facilities are also being set aside for conjectured production by genetically engineered *Escherichia coli*. A new plant will be built at Searle's High Wycombe, UK, factory.

"But this project is purely for research and development", says Searle's director of research and biological development at High Wycombe, Dr Brian Richards. "It will cost us £6.75 million, and we don't intend to make a profit out of it".

Searle's increased fibroblast interferon production will be supplied to the Anderson Hospital of the University of Texas for clinical tests of the drug in cancer control. The trial has been designed to match an earlier one with leukocyte interferon; it is the first on cancer patients with interferon from fibroblasts, although there has been a successful trial on patients suffering from herpes keratitis of the eye (where the application was external). The difference between fibroblast and leukocyte interferon is not just that they come from different kinds of cells. By several laboratory criteria they also differ in structure. What is not yet clear is whether this will affect their therapeutic value.

Two-thirds of the space in the new plant

will be set aside on a conjecture: that within 18 months strains of genetically engineered *Escherichia coli* will exist which can compete with fibroblasts in interferon production.

"Charles Weissmann in Zurich has produced *E. coli* which manufacture one or two molecules of interferon per cell," says Dr Richards. "I'm quite satisfied he has the right gene product, but the problems are going to come in trying to improve the efficiency."

Searle has in fact constructed bacteria carrying other genes that are expressed with high efficiency — "we have cells producing haemagglutinin at 1% of total protein of the cell" says Richards. Enough is not yet known about expression efficiency to predict whether such bacteria containing the interferon gene in place of the haemagglutinin gene, would be equally efficient, but apparently it gives "cause for hope".

Searle's current interferon production is roughly one quarter of world production, including leukocyte interferon, he estimates. So with the increased fibroblast production alone, world interferon production will increase two or three-fold. "Ultimately costs might fall by a factor of 100 to 1000", Richards estimates. Interferon costs would then be on a par with those of antibiotics.

NEWS IN BRIEF

ESA chooses Hipparcos

THE Scientific Programme Committee of the European Space Agency decided last week to fund the astrometry mission Hipparcos as the next ESA mission after Exosat which is to be launched in 1981. Designed to improve the measurements of stellar positions by two orders of magnitude, the satellite will be launched by Ariane in mid-1986, and placed in a geostationary orbit for its lifetime of two and a half years. The total estimated cost of the project is 139.3 MAU (\$185 million).

Hipparcos was the Programme Committee's final choice in spite of a recommendation by the Scientific Advisory Committee to fund a dual mission consisting of experiments to measure the Earth's magnetospheric tail and a deep space flyby of Halley's comet. By a vote of 10 votes for and one abstention, the 11 member committee, consisting of delegates from each ESA country, decided to overturn the Scientific Advisory Committee's recommendation.

No evidence found of link between saccharin and cancer

No evidence of a positive link between the consumption of saccharin and an increased incidence of bladder cancer has been revealed by either of two studies whose results were published last week. One was carried out by Harvard University's School of Public Health and the other by the American Health Foundation.

The scientists who carried out the study, which involved surveys of the dietary habits of 592 and 367 bladder cancer patients respectively, point out that these results have two possible implications. Either saccharin does not cause cancer; or that its effects are too small to be identified by such studies.

However, writing in the *New England Journal of Medicine*, Dr Robert Hoover, director of a separate study carried out by the National Cancer Institute, states that "the evidence is that little, if any, current bladder cancer is due to the consumption of artificial sweeteners at the doses and in the manner in which sweeteners were commonly consumed in the past."

The Harvard study, reported in last week's issue of the journal, came to the conclusion that artificial sweeteners posed "little or no excess risk of cancer of the lower urinary tract", although pointing out that, since the latency period for cancer due to exposure to toxic chemicals can be 30 to 50 years, it will be some time before definite conclusions can be reached.

The Food and Drug Administration has proposed a ban on the use of saccharin following evidence that it can cause tumours when administered to animals at

high doses. However, the ban was held up by Congress in 1977 until further information about saccharin's effect became known.

Academy report attacked on solar energy predictions

A RECENT report published after four years of study by the Committee on Nuclear and Alternative Energy Systems of the National Academy of Sciences has been attacked for severely over-estimating the costs of developing solar energy as a major source of power — and hence over-stating the need to develop nuclear and coal technologies.

In its report, prepared for the Department of Energy at a cost of \$3 million and subject to some bitter internal disputes, the committee states its conclusion that solar energy "will probably not contribute much more than 5% to energy supply" in the US before the end of the century, at least not without massive government intervention to penalise the use of non-renewable fuels.

This conclusion, however, is being strongly contested by environmentalists, who last year persuaded the Carter administration to commit itself to policies for supporting solar energy research and development designed to meet 20% of the nation's energy needs by the year 2000.

In a letter to the *New York Times*, Gus Speth, chairman of the White House's Council on Environmental Policy, says that as far as he has been able to discover, the committee's estimate that the cost of reaching the administration's target was a prohibitive \$3,000 billion was the result of a "back-of-the-envelope calculation", based on assumptions such as all capital equipment being constructed at today's prices.

Reworking the calculation with declining prices predicted by the committee's Solar Resources Group reduced the cost by a factor of almost three, he said.

A spokesperson for the academy said last week that a reply to the CEQ's charges was being prepared and would be submitted to the *Times* for publication.

Canada appoints new science minister

JOHN ROBERTS, cabinet minister responsible for cultural affairs in Canada's last Liberal government, has been appointed Minister of State for Science and Technology in the new cabinet announced last week by Prime Minister Pierre Trudeau after the Liberal Party's success in the general election.

Roberts will also carry the portfolio of Minister of the Environment. This has disappointed officials in scientific and

professional societies who had hoped that the new government would, like its Conservative predecessor, appoint a minister with sole responsibility for science and technology.

However the Liberal Party has indicated that it intends to make the creation of a strong research and development effort in Canada one of its top economic priorities. An outspoken economic nationalist, Mr Herb Gray, who has been appointed Minister of Industry, Trade and Commerce, said last week that Canada's "disturbingly low level" of R&D expenditure was connected with the high level of foreign control of Canadian industry. Canada's Foreign Investment Review Agency, created in 1974 at Gray's suggestion, will have its mandate extended to investigate the R&D performance of such companies.

Livermore safety report criticised

OFFICIAL reports of "minimal damage" at the Livermore nuclear weapons laboratory from two earthquakes last January were criticised severely last week. Two structural engineers, John Rutherford and Gary Gray inspected the plutonium building where up to 300 lbs of plutonium are stored and described the damage as "severe". A wall had moved during the tremors (the largest of which measured 5.5 on the Richter scale) and it was in danger of collapse.

Friends of the Earth claimed that a big earthquake of the order of 6.5 on the Richter scale could cause a fire which would burn the plutonium causing it to disperse over a wide area in a radioactive cloud.

Dr John Gofman, of the University of California, and a former director of the laboratory, called the Livermore laboratory "a public health hazard". "There is a conceivable possibility of fire and the plutonium burning thus becoming airborne dust. To say, as the officials have, that nobody would be killed is nonsense", he said.

Sakharov vote avoided

THE Russian Academy of Sciences has avoided putting to the vote the expulsion of Dr Andrei Sakharov from its ranks. Two corresponding members of the Academy had proposed a vote but last week were persuaded to withdraw their proposal. Had a vote been forced, it would have taken place in secret and required a two thirds majority to succeed. There is only one precedent for the expulsion of a member of the Academy, and that was of someone whose original election was based much more on political grounds than on genuine scientific achievement.

FEATURES

Swedes vote on their nuclear future

On 23 March the people of Sweden will be voting in a referendum on nuclear power policy. **Wendy Barnaby** writes from Stockholm that the exercise is intended to meet party political rather than major policy objectives

AFTER more than seven years of increasingly acrimonious public debate about nuclear power, nearly all political parties are badly split on the issue and energy policy is totally stranded. Popular support for a referendum was building up at the end of 1978, encouraged by the anti-nuclear Centre Party and various environmental groups, but it was staunchly opposed by the other parties until the accident at Harrisburg. It then became obvious to the leader of the Social Democratic opposition, Olof Palme, that his only chance of winning the general election scheduled for September, 1979 was to about-face and support a referendum.

Faced with a parliamentary majority in favour of a referendum, the minority Liberal party government (which had already tabled an energy bill incorporating 12 nuclear reactors) had no choice but to go along with it as well. The Centre Party and the Communists, also anti-nuclear, argued that the referendum should be held at the same time as the election, but they were overruled by the combined forces of the Social Democrats, Liberals and Conservatives, all of whose leaderships are pro-nuclear, but whose backbenchers and rank and file are split. These parties would have been embarrassed to have their internal disagreements aired during the election campaign. So the referendum was scheduled for March, 1980 — possibly in the hope that the intervening northern winter and the spectre of oil rationing might have a salutary effect on the population's attitudes. (Sweden depends on imported oil for 70% of its total energy consumption, which amounts to a little more than 400TWh a year. Electrical energy, which makes up 90TWh of this, is produced mainly by hydropower. Nuclear power provides 25% of electrical energy; the rest comes from oil).

In the event, Olof Palme's about-face did not win him the election: the bourgeois bloc won with an overall majority of one seat. Nor were nuclear issues prominent in the campaign. They would, it was said, be discussed once and for all before the referendum. But it is clear that the referendum will not be the final curtain of the nuclear drama. It will simply set the stage for another long act.

After the formation of the new bourgeois government (a coalition of the Centre, Liberal and Conservative parties

with Centre leader Torbjörn Fälldin as Prime Minister), the referendum questions had to be drawn up. The party leaderships were divided essentially into two camps: the Communists and the Centre party opposing nuclear power, and the others in favour. But to draw up two alternatives would have meant that the Social Democrats backed the same alternative as the Conservatives. This was politically unpalatable to the Social Democrats, who feared they would lose voters if they were seen to be aligned with the Conservatives on such a crucial issue. They therefore wrote into their referendum manifesto that all power production should be state-owned: not a revolutionary demand, as about 85% of it is state-owned already. As expected, however, the Conservatives could not go along with this, so the pro-nuclear side split into two factions.

As a result of this political face-saving, the voters must now decide between three options. Option three, supported by the Centre and Communist parties, Christian organizations and a variety of environmental and radical fringe groups, advocates phasing out the six reactors presently in operation over a period of ten years, and intensifying energy-saving and investment in renewable energy sources. Option two, supported by the Social Democrats and Liberals, advocates "phasing out nuclear power at a pace possible, taking into account the need for electric power for the maintenance of employment and welfare." In practice, this pace would mean, according to option two, using the six reactors now in operation as well as the four ready for loading and the two under construction — a total of 12 — for their lifetimes (about 25 years), but not building any more.

Option one, supported by the Conservatives and industry, also advocates (in identical wording to option two), "phasing out nuclear power at a pace possible taking into account the need for electric power for the maintenance of employment and welfare". This pace would also mean using all twelve reactors. Officially option one also vetoes building another generation of reactors, but its backers make no secret of the fact that this is written in because it would be unwise to declare their enthusiasm for nuclear power in the present political climate. Option one is generally regarded as being more pro-nuclear and more likely to go ahead with



Two Swedish Prime Ministers: former Social Democrat Olof Palme (left) switched to supporting the referendum against his party line. New Centre premier Torbjörn Fälldin (right) is anti-nuclear, but could not implement an anti-nuclear decision

uranium mining in Sweden and breeder reactors than option two.

Having three options produces a situation fraught with difficulties. Although option three has been labelled the 'no' side, it is in fact advocating a further use of nuclear power — for 10 years. Options one and two have also labelled themselves 'phasing out' options, although they advocate a further use of nuclear power on a much larger scale than option three. The debate is not in fact about a 'yes' or a 'no' to nuclear power, but the number of reactor-years nuclear power should be allowed to stay. The public is not faced with a clear-cut choice. Another obvious difficulty with three options is how to interpret the results. The new government had a hard time appointing an Energy Minister, but they finally engaged the services of a non-party judge, Carl-Axel Petri. According to him, the support for options one and two will be counted together against option three. Option three must therefore poll more than the other two combined if it is to win. The leader of option three's campaign, Lennart Daléus, has said his option ought to be considered the winner if it polls more than either of the other two options singly. (The latest public opinion poll shows option one being supported by 25% of the population, option two by 34%, option three by 35%, with 6% undecided).

Although the referendum is advisory, all parties have said they will follow its result. But whatever the result, there will be political problems. It seems certain that options one and two will together poll more than option three. For Prime Minister Fälldin, opposition to nuclear power is a moral stance, and it is doubtful whether a referendum result could give him a moral absolute to preside over a government taking another six reactors into operation. In the unlikely event of option three's winning, there would be other political problems. The Centre and Communist

parties do not have a parliamentary majority and would therefore be unable to enact the measures the people had voted for. This would probably lead, sooner or later, to a government crisis and new elections.

While the different factions squabble, the Swedish Council for Planning and Coordination of Research is trying to produce impartial, informative material. It has engaged pairs of authors with different opinions on energy questions to argue their cases on eleven topics, including how to store nuclear waste, how to heat houses, nuclear power and nuclear weapons, and sun, wind and biomass. Each of the pairs has a mediator whose task it is to clarify where the authors agree and disagree.

Whatever the outcome of the referendum, the campaign has demonstrated a lot about the treatment of a complex scientific and technical issue in a democracy. Rational argument about energy has been relatively unimportant. What has been important is politics.

Supporters of option three oppose nuclear power largely, it seems, because they see it as a symbol of many unattractive features of modern, post-industrial life: individual isolation, large-scale technology, powerlessness of the individual to determine living conditions, and so on.

Supporters of options one and two talk about the waste of investment if the country does not use reactors it has built. What they envisage is essentially a continuation of Sweden's post-war history: an economically-expanding society using the latest technology to secure personal welfare. □

THE Swedes estimate that the International Nuclear Fuel Cycle Evaluation (INFCE) has increased their chances of obtaining United States approval for reprocessing spent fuel from their reactors. Because they use American enrichment facilities, they are currently forbidden to reprocess spent fuel under the US Nuclear Non-Proliferation Act of 1978.

After the INFCE discussions, however, the US may well give Sweden permission — however guarded — to send spent fuel to existing reprocessing plants and leave the plutonium there.

All Swedish decisions on nuclear matters are in abeyance at the moment, pending the referendum. Irrespective of the result, however, nuclear policy will probably develop along the lines of rejecting a national facility for reprocessing and not insisting on having the plutonium processed abroad sent back. Sweden might well sell its plutonium to the countries which reprocessed it. Such a development would dovetail nicely with some sort of American permission to reprocess.

The two countries are negotiating a new agreement on nuclear cooperation, and it will be as part of this agreement that the reprocessing question will be raised.

Drug innovation— what's slowing it down?

The research and introduction of new drugs has been slowing down in the UK and the US. At the same time, government regulations on the safety of new drugs have increased. But **Fred Steward** and **George Wibberley** argue that there is not a simple relationship between these two trends

THE INFLUENCE of government safety and efficacy regulations on pharmaceutical innovation has become a prominent issue in relations between the industry and the British government. Last April the Association of the British Pharmaceutical Industry (ABPI), in its "election manifesto", criticised the government for producing "a cumbersome and expensive regulatory edifice, much of which serves little useful purpose so far as the well-being of the public is concerned". Vice-President Dr Peter Main singled out as a fundamental concern "the effect that the ever-increasing delay caused by regulations relating to the testing of new products was having on pharmaceutical research and innovation". The Association's news sheet has continued to give emphasis to the issue, accompanied by headlines such as 'Murder by Regulation' and 'Take off the Cuffs'.

Last September the new UK Health Secretary, Mr Patrick Jenkin expressed his concern that controls for reasons of safety, quality and efficacy "can reach a point where they are so thorough, so pervasive and so foolproof that everything comes to a full stop". He was anxious that "unnecessary impediments to innovation should be removed".

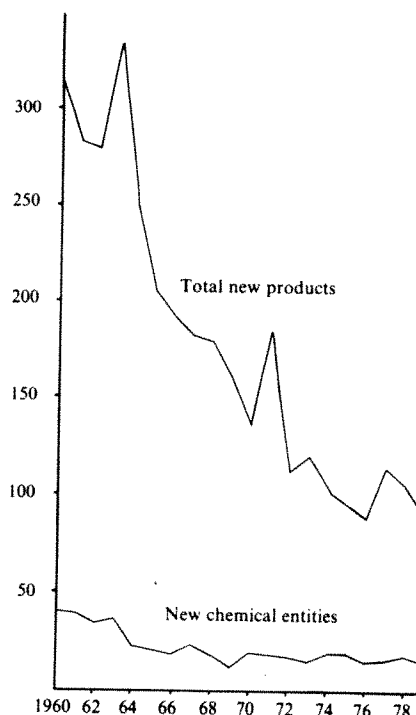
These views on the drug industry have been accompanied by mounting pressure against government legislation by other sections of British industry, and they have been anticipated in many ways by apparently similar arguments in the United States. The recent federal review of industrial innovation attributed negative effects to laws concerning health, safety and the environment, a conclusion much in tune with President Carter's commitment to "reduce, rationalise and streamline the regulatory burden". Attention has been focused throughout the 1970s on the impact of such controls on the performance of the drug industry, culminating in the introduction of a variety of proposed drug regulatory reforms in

Congress during 1978 and 1979.

The time is therefore appropriate to ask what exactly are the effects of regulation on innovation in the drug industry? Two points need to be made at the outset: much of the data and analyses have looked primarily at the US situation; and there are factors other than the legal environment influencing drug innovation. The Pharmaceutical Innovation Group at Aston University has been gathering data on drug innovation in the UK with the aim of evaluating the influence of regulation compared to other factors.

The annual rate of introduction of brand-name pharmaceutical products which doctors in general practice can prescribe in the UK has declined substantially from 1960 to the mid-1970s (Fig 1). This includes duplicates, new formulations, dosages and combinations of existing drugs. New chemical entities (NCEs) — products containing a new chemical substance — comprise only a small proportion of all new products. The annual rate of introduction of NCEs into

Figure 1: pharmaceutical products introduced into the UK 1960-79



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the UK market also declined in this period from about 30-40 to 15-20 per year; this rate remained reasonably steady throughout the 1970s.

Similar trends have been observed elsewhere. An analysis by Erika Reiss-Arndt of the annual rate of introduction of NCEs into the world market showed a decline, mainly in the late 1960s, from nearly 100 in 1963 to 65 in 1973. The rate of introduction of NCEs into the US market, according to the data of Paul de Haen, shows an even sharper drop, from over 70 in 1960 to less than 20 in the 1970s.

The general decline in the rate of introduction of new drugs in the 1960s coincided with the introduction of stricter requirements in the UK and the US for the pre-market testing of new drugs. In the US the 1962 Kefauver-Harris amendments asked for evidence of efficacy in addition to evidence of safety already needed under the 1938 Food, Drug and Cosmetic Act. In the UK, a system for evaluating drug safety was introduced after the thalidomide disaster. The non-statutory Committee on the Safety of Drugs chaired by Derrick Dunlop began operating in 1964 and was replaced by a statutory licensing system involving the Committee on the Safety of Medicines under the 1968 Medicines Act. This put emphasis on efficacy as well as safety.

There are two particular difficulties in assessing the relationship between these regulatory changes and shifts in the rate of innovation. The first is that the large size of the US market and the importance of the US industry means that domestic regulations affecting them may have wider international repercussions; and the second is the fundamental problem of separating the influence of regulatory and non-regulatory factors on the pattern of innovation.

If there is a need for government intervention to prevent the marketing of inadequate drugs, then as a consequence of established control mechanisms, a decline in the introduction of inadequate products would be expected.

The greatest decline in new drugs in the UK has been of those containing an active ingredient already available in some other product. In the past, the medical profession has criticised the proliferation of similar and unnecessary combination products. The Committee on the Review of Medicines recently emphasised their lack of therapeutic value. There have been few claims that innovation in the formulation of existing drugs has been inhibited in recent years. Instead, the decline in the introduction of products based on existing drugs appears to fulfil the explicit objectives of regulatory policy.

The fall in the rate of introduction of NCEs is more difficult to analyse. By definition they possess some chemical novelty, but this is no guarantee that they all offer benefits in terms of safety or effectiveness. In the US, the Office of

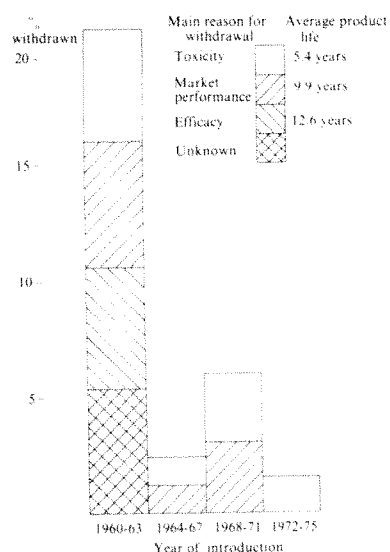


Figure 2: withdrawal of new chemical entities introduced in the UK 1960-75

Health Economics has obtained assessments by clinical experts of the therapeutic significance of NCEs introduced between 1958 and 1970.

Studying the introduction of NCEs in the UK, we found that the proportion in the bottom category of 'little or no advantage' dropped from 32% in 1960-63 to 18% in 1967-70. This included products which failed to meet the basic minimum standards set by the control system. Again, this decline appears to fulfil policy objectives. A classification by the Food and Drug Administration (FDA) of NCEs introduced in the US also showed a decline in the bottom category, representing 'no therapeutic gain', from 75% of 1961-1962 introductions to about 40% of introductions in the late 1960s and early 1970s.

It is remarkable that in the wide range of studies on regulation and innovation, relatively little effort has been devoted to exploring more thoroughly the degree to which the primary intended effects of regulatory change have been fulfilled. We have examined the rate of failure as shown by subsequent market withdrawal of NCEs introduced into the UK. The lack of attention given to this was illustrated early last year when the then Health Minister could not give any information in response to a parliamentary question about the extent of, or reasons for, the withdrawal of drugs from the UK market.

An NCE may be withdrawn for a number of reasons, including toxicity problems, inadequate efficacy and poor market performance. Our analysis of the withdrawal of NCEs introduced in the UK from 1960 to 1975 (Fig 2) shows a very much lower overall rate of withdrawal of NCEs introduced from 1964 onwards. Over 20% of the NCEs introduced in 1960-63, before the Committee on the Safety of Drugs started operation, were subsequently withdrawn from the market. Recent introductions may distort these

figures but the trend shown bears out the view that fewer drugs which ultimately failed reached the market after the establishment of stricter control.

The persistence of NCEs with unacceptable toxicity shows the importance of more effective surveillance of drugs when they enter medical practice. Some of the decline in the rate of introduction of NCEs in the UK therefore fulfils the goals of drug control policy.

The 1960s decline in the rate of NCE introduction, however, cannot be accounted for in this way. Increases in cost and length of time involved in discovering and marketing new drugs are also factors.

We measured the time from patent or publication, to the marketing of a new drug in the UK. Averaging about three years in the early 1960s, this rose to about 7.5 years in the early 1970s and remained then reasonably steady until a rise to nearly nine years in 1978-79. These increases have often been attributed to the demands of regulatory bodies, and it has been suggested that the overall decline in the rate of NCE introduction is a consequence of drug regulation.

But what are the other possible influences on the time and expense of drug research? A number can be suggested. The identification of many biologically important chemicals shortly after the war may have created a fruitful basis for innovation, for a period, but subsequently showed diminishing returns. Changes in the attention given to certain diseases could also have significant effects on the discovery and evaluation of new drugs. The complex aetiology of diseases such as cancer poses problems which cannot be resolved simply by expanding the screening of new compounds. The complexity of cardiovascular diseases again illustrates the difficulty of finding clear cut cures. There is, therefore, a need for more extensive evaluation of the effectiveness and toxicity of drugs intended for long-term administration.

Some of the areas in need of major innovation, such as inflammatory disease, raise the particular problem of suitable animal models for screening purposes. All these factors point to more complicated, time-consuming and expensive drug research. Another factor may be the rising expectation of researchers for more sophisticated and expensive equipment.

An analysis we have undertaken of the diseases for which new drugs are introduced in the UK shows a shift from infectious diseases in the mid-1960s to cardiovascular disease in the mid-1970s. Detailed information on pharmaceutical R&D in the UK is not readily available, but some figures have been published for the US by the Pharmaceutical Manufacturers Association. These show that in 1977 35.9% of total R&D expenditure was incurred by discovery-oriented work of synthesis, extraction, biological screening and pharmacological testing. Clinical

evaluation took 22.5%, toxicology and safety testing 9.2% and regulatory preparation and submission 3.3%.

Although the rate of introduction of NCEs stabilised during the 1970s, it is becoming harder to discover new molecules of real therapeutic importance. Of 83 NCEs approved by the FDA between 1974 and 1978, 62% were considered to offer moderate or important gains. This contrasts with only 33% of the 45 new molecules in pending new drug applications in March 1978.

Whatever the cause of increases in the cost and time of drug innovation, the consequence has been a decline in the "productivity" of R&D investment.

The influence of regulations in the US has received considerable attention, much of it concerned with different rates in the introduction of NCEs in other countries. William Wardell, in particular, has documented in detail a persistent "drug lag" in the US from 1963 onwards, shown by the high number of NCEs introduced in Britain but not marketed in the US, or only marketed there some years later.

Although the marketing strategy of a particular company determines the country in which the drug is introduced, many of the differences between the US and UK arise from differences in regulatory approach. There have been conflicts between the FDA and its critics over the therapeutic significance of the 'lag'. Some have argued that important new drugs such as sodium cromoglycate were excessively delayed while others have replied that the proliferation of very similar drugs, such as the non-steroidal anti-inflammatory drugs, has been avoided. Wardell has criticised the administration of the regulations and not the existence of the 1962 Amendments.

Perhaps an acknowledgement of all these points lies in the FDA's new fast-track approval process which allows important innovations to move more quickly through the regulatory system. The mean approval time for 'promising therapeutic advances' in the year beginning October 1978 was 19.4 months compared with an average of 39.2 months for those of 'little or no therapeutic gains'. But the speedier British system carries disadvantages. Of the four NCEs introduced in the UK since 1964 that were later withdrawn on the ground of unacceptable toxicity, three (ibunefac, practolol and alclofenac) were never introduced in the US.

Any comparison of the risks of adverse drug reactions with the risks of delay in the introduction of important new drugs, presents serious difficulties. The assumption that reduction in the risk of adverse effects from drugs must be accompanied by an increase in the time taken for their introduction is not necessarily valid. Labels for regulatory systems such as 'strict' or 'fast' should be used cautiously especially when comparing

one system with another.

The underlying weakness of many studies on the relationship between drug regulation and innovation is that they treat R&D as a "black box" and use highly aggregated measures of innovative output. Although correlations have been observed between factors such as regulatory change, development time, research costs, and the rate of innovation, they have not been accompanied by comprehensive explanations of what changes have been taking place within the research process.

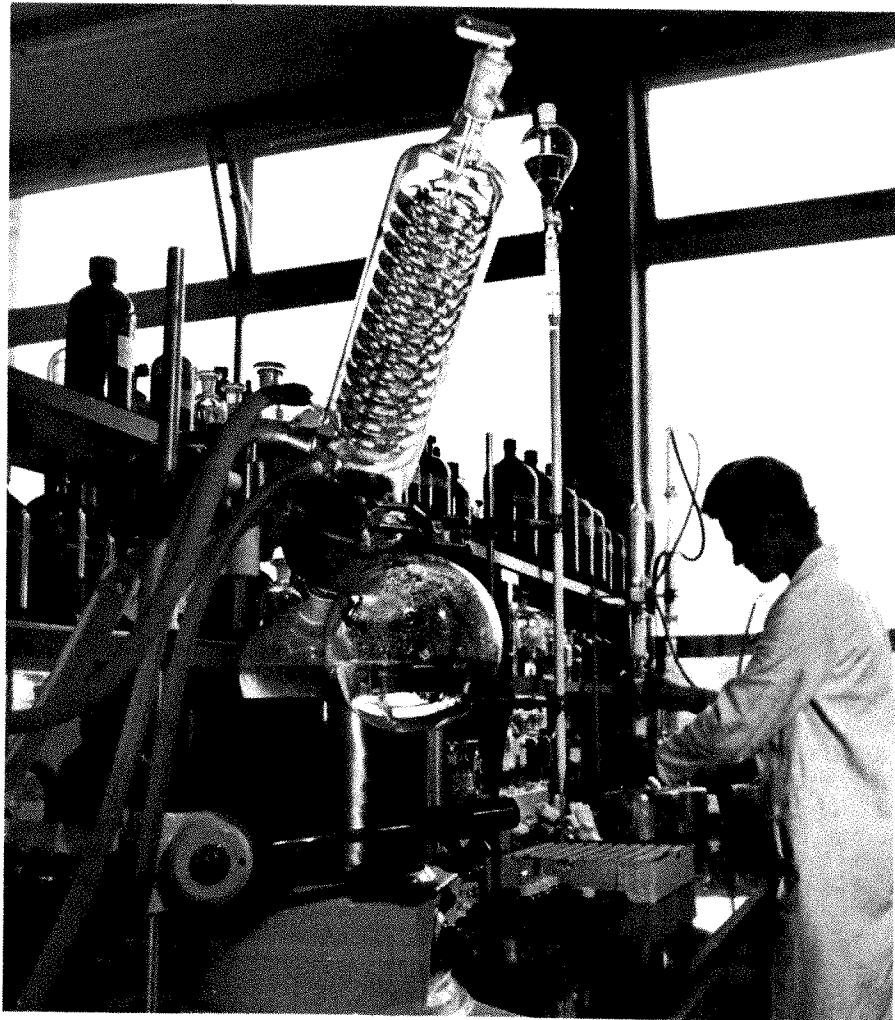
The decline in R&D productivity has meant changes in strategy. The proportion of expenditure on "basic" as opposed to "applied" research in the US drug industry fell steadily over the decade 1968-78 from 15.4% to 11.4%. British data are not precise enough to detect such a trend. There also seems to have been a move away from less economically significant disease, such as those affecting small numbers of people, or those prevalent in poorer parts of the world. Our analysis of the introduction in the UK of drugs for uncommon illnesses as a proportion of all new drugs shows a drop from 7.1% in 1960 to 4.7% in 1971-76. These changes are taking place in the context of companies concentrating on fewer therapeutic areas. Jerome Schne

has shown that those US drug firms which concentrated research on fewer areas produced an average of 1.3 NCEs in 1972-75 while those which diversified their research generated an average of only 0.67.

These changes in R&D strategy may have major social consequences. The shift from basic research may well result in just the opposite effect to the closer relationship between basic and applied science. Changes in the therapeutic categories receiving attention may mean a neglect of important breakthroughs in less commercially attractive areas. Although such research decisions have widespread repercussions, they are not subject to any direct form of social control. The proposition that less, rather than more, government intervention is required in relation to drug innovation is at least an arguable one.

The precise relationship between regulation and innovation has yet to be established, and it is unlikely to be determined by further studies which attempt to quantify the adverse effect of regulation but do not examine the full range of factors which may influence drug innovation. We are exploring research and innovation in the drug industry with this perspective in mind. □

Evaporation apparatus for concentrating drug solutions in use at the Wellcome Laboratory of Drug Metabolism, Beckenham, Kent, UK.



NEWS AND VIEWS

Neural tube defects: towards prevention and understanding

from Gill Morriss

ANENCEPHALY and spina bifida aperta are open neural tube defects (NTD) with an overall population frequency in Britain of 1 in 500 and 1 in 400 respectively, although there are marked geographical variations¹. Their aetiology is not well understood, so that prevention has, until now, been possible only through antenatal diagnosis (alpha-fetoprotein testing) and subsequent abortion.

In a recent clinical trial the expected incidence of NTD was greatly reduced after prospective mothers at high risk were given vitamin supplements before conception and during the early weeks of pregnancy. If these results hold up in more extensive tests, true prevention will become a possibility in many cases.

These results are also interesting scientifically as they indicate that environmental (in this case nutritional) factors may have a much greater weighting in an individual's predisposition to NTD than was formerly realised. It is therefore possible that relatedness to an affected individual increases predisposition to the same malformation through common environmental factors as well as through common genes. The interaction between environmental and genetic factors has also been highlighted in recent work on a mouse model for NTD.

Before discussing these reports in more detail, it is useful to consider the concept of predisposition to malformation as proposed by Carter¹. The combined environmental and genetic factors predisposing an individual to the risk of malformation during development ('individual' here refers to the embryo) are assumed to be normally distributed in the population for any specific congenital malformation. The threshold at which an individual's total liability will result in malformation, lies well away from the mean population level of predisposing factors. This distribution is indicated by the solid line in Fig. 1. There are subpopulations within the general population which have an increased risk. For example, an increased predisposition can be predicted for the relatives of an affected individual (the index patient). According to Carter's model, the risk will depend on the number of predisposing

genes they have in common with the patient: 50% for first degree relatives (siblings and offspring), 25% for second degree relatives (nephews and nieces) and 12.5% for third degree relatives (first cousins). Increased risk within a subpopulation is indicated in Fig. 1 as a shift of the distribution curve to the right, so that a larger number of individuals have above threshold levels of predisposing factors (broken line). (Fraser² has used a similar diagram to illustrate the shift in the normal distribution curve to the right when two teratogens interact additively: genetic/environmental interactions can also be depicted in this way.)

Evidence for the importance of genetic factors in the causation of NTD comes from family studies and from racial variations which are at least partially maintained after migration. Evidence for the importance of environmental factors comes from studies on the effects of social class, maternal age and birth order, seasonal variation and so on³. In reality, it is impossible to assess the relative importance of genetic and environmental factors, nor even to distinguish between them adequately. Families and close-knit communities tend to have many cultural factors in common and nutritional habits may be maintained after migration. The reasons for the marked geographical variation are similarly obscure. Even though the reasons for differences in predisposition are so poorly understood, the increased risk of recurrence within a family is well documented: for instance, in the South Wales study of Carter, David and Laurence⁴, 5.2% of siblings of an NTD index patient were themselves affected, compared with an incidence of 0.77% in the general population of that region. (The latter figure is itself high compared with other regions.)

It was on the basis of these studies that Smithells *et al.*⁵ were able to select a high-risk group of potential mothers to test the hypothesis that the social class gradient in incidence of NTD was due to nutritional factors. Their own earlier studies had indicated that red cell folic acid and leukocyte ascorbic acid (vitamin C) deficiencies in early pregnancy might be correlated with NTD⁶. 175 women who had previously given birth to an NTD infant, and who wished to become

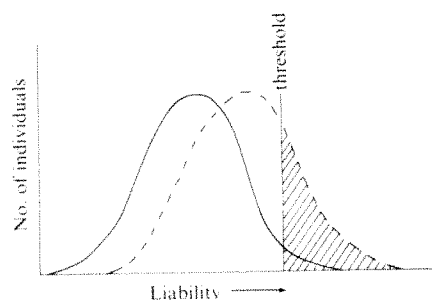


Fig. 1 Shaded area represents malformed individuals (see text). Modified from Carter¹.

pregnant again, were included in the study. They were given a multivitamin and iron preparation for at least 28 days before conception, continuing at least until the date of the second missed period, that is beyond the normal time of neural tube closure, which is completed 25 days after conception. (In view of the earlier suggested correlations with folic and ascorbic acid deficiencies, it is unfortunate that such a blanket preparation was used.) Only one of these mothers has produced a further affected infant. Fifteen mothers had an even higher risk than the rest of the group by virtue of having had two previous NTD infants; none of these had a further affected child. (The figures include 26 continuing pregnancies with normal amniotic fluid alpha-fetoprotein levels.) The recurrence rate of 1 in 178 infants (3 twin pairs) could be slightly higher than the 0.6% calculated, since there were 10 unexamined spontaneous abortions. Nevertheless the result is encouraging when compared with the recurrence rate in the control group (no vitamin supplementation), which was the expected number, 13 of 260 infants (5.0%).

Caution is essential at this stage: it is salutary to recall that the suggestion that anencephaly and spina bifida were "usually preventable" by avoidance of blighted potatoes, was also supported by impressive statistics⁷. It is therefore very much to be hoped that the results of the nutritional supplementation study will be confirmed by extension of the trial to larger numbers of patients.

The concept of genetic and environmental factors affecting the teratogenic threshold has been highlighted recently in studies on the curly-tail mouse^{8,9}. This is a

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mutant with an incidence of spina bifida, exencephaly (\equiv anencephaly) and related axial defects of around 50%. In outcrossing experiments with BALB/c and A strong random bred strains, the incidence of the curly-tail phenotype was reduced to 24% and 8% respectively (F_1 female backcrossed with curly-tail male parent), that is, the expression of the (recessive) gene was modified by the maternal genetic background.

The expression of exencephaly, unlike that of spina bifida, was strongly sex-linked, with an overall ratio of 0.25 male:female. A similar bias has been observed in another mouse mutant with a high incidence of exencephaly¹⁰, and the same has long been recognised in human anencephalics (ratio 0.34 in the South Wales study of Carter, David and Laurence⁴). These observations suggest that anencephaly involves a sex-linked gene-gene interaction, acting either on morphogenesis or on subsequent viability. Why this should be so for anencephaly and not for spina bifida is unclear, but it is apparent from morphological and ultrastructural observations that the sequence of mechanisms involved in morphogenesis of the cranial part of the neural tube is more complex than that of the spinal region¹¹. The striking morphological difference between the two regions is illustrated in a five-somite rat embryo in Fig. 2.

Excess vitamin A is well known as a teratogen which can affect neural tube closure when administered before or during the period of neurulation. Seller *et al.*⁹ have compared the effect of various dose levels of vitamin A given to curly-tail and A strong mice on day 8 of pregnancy. The effect of interaction of this environmental teratogen with the NTD-disposed curly-tail genotype was a shift of the teratogenic response to the right, that is, NTD resulted from lower dose levels in curly-tail compared with A strong mice. The higher dose levels, merely teratogenic in A strong, were lethal in curly-tail. This is a nice example of genetic/environmental interaction.

An intriguing result from another set of

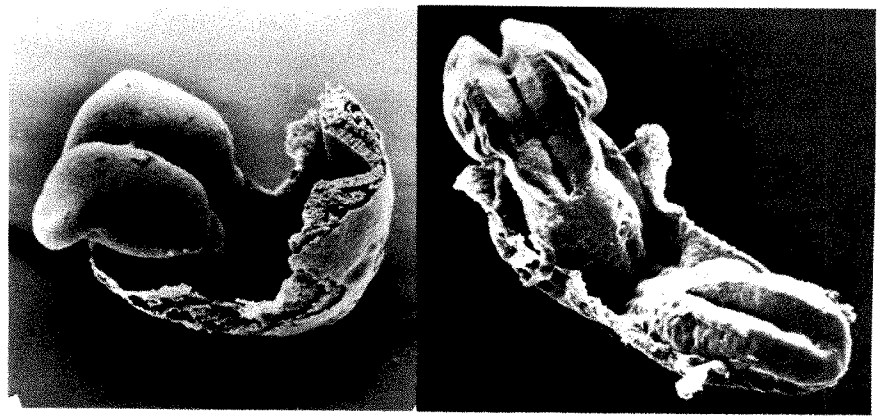


Fig. 2 Scanning electron micrographs of 5-somite (left) and 9-somite rat embryos. At 5 somites the cranial neural folds are relatively large, convex structures compared with the V-shaped neural groove of the spinal region. By the 9-somite stage, when neural tube closure has begun in the cervical region, the shape of the neural folds is similar in both cranial and spinal regions.

experiments in this series was the observation that administration of the two lower dose levels of vitamin A (5 mg kg⁻¹ and 10 mg kg⁻¹) to curly-tail mice on day 9 resulted in a significant decrease in the proportion of malformed fetuses (33 and 36% respectively, compared with 54% in the sham-injected controls). This result is not to be compared with the effects of vitamin supplementation in high risk women — in the animal experiment we are considering a paradoxical genetic/environmental interaction, since administration of the same dose levels one day earlier had a potentiating effect on the genetic predisposition to NTD.

One must emphasise here that there are many possible interpretations of these observations, particularly since the neural tube of normal mice has completed closure by day 9, and it is usually assumed that open NTD are the result of failure of closure of the tube. Indeed, many previous studies on excess vitamin A have failed to induce defects following injection on day 9¹². It may be that NTD in the curly-tail mouse are associated with a propensity for delayed neurulation, and that vitamin A administered on day 9 interacts with the genetically-induced abnormal morphogenetic mechanisms in such a way as to increase the number of still open neural tubes which are able to complete closure at this late stage.

However, the possibility that there is secondary reopening of a closed tube cannot be altogether ruled out. At the region of closure (the dorsal midline) the closed neural tube and the surface ectoderm above it are both thin epithelia which are very easily broken during preparative procedures such as paraffin embedding, suggesting that a potential weakness may exist here in the living embryo. This said, it must be recognised that the weight of experimental evidence supports the assumption that open NTD usually result from failure of the neural tube to complete closure. This has been demonstrated in the case of excess vitamin A, for instance, by the addition of retinol

or retinoic acid to the culture medium in which neurulating rat embryos are developing¹³. Unfortunately vitamin A is of limited usefulness as a teratogen if one is searching for mechanisms at the cellular or molecular levels, because the molecular nature of its effects on morphogenesis is still obscure.

As one would expect in a system involving curvature of an epithelium, neural tube closure can be prevented by culturing embryos in the presence of substances such as cytochalasin B, which affect the contractile properties of actin-containing microfilaments¹⁴. But organised breakdown of microfilament bundles occurs in the most lateral cells of the neural epithelium in the last stage of curvature towards the midline before fusion during normal development; if this particular breakdown of microfilament bundles does not occur, neural tube closure again fails¹⁵. These contrasting observations illustrate the complexity of the process of neurulation, and make it clear that no single factor or group of factors can explain the cause and genesis of NTD.

Although the curly-tail mouse was first proposed as a model for human NTD twenty-one years ago¹⁶, no attempt has yet been made to investigate the morphogenetic basis of the observed abnormalities. The studies discussed above confirm the potential of this mutant, and demonstrate the interaction of genetic and environmental factors, but they do not provide any insight into the morphogenetic mechanisms underlying the NTD. Such insights will come only through painstaking observations of the embryos themselves, before and during the process of neurulation. Wilson and Finta¹⁷ have investigated the genesis of spina bifida in the splotch mouse mutant in this manner, and have found abnormally large numbers of gap junctional vesicles in the day 9 neural epithelium at the level which fails to form a closed tube. They discuss the possibility that these vesicles may be the visible result of an earlier process which has

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interfered with normal cellular contact mechanisms such as intercellular communication or adhesion; this interpretation is supported by the observation that the neural epithelium subsequently develops a looser structural organisation

than normal¹⁸.

Morphogenetic patterns and mechanisms in postimplantation embryos present serious problems of analysis because of their enormous complexity. However, we must come to terms with

these difficulties if we are to improve our ability to interpret and predict causes and origins of human malformations, and to be able to extrapolate from the results of experimental work on laboratory animals to man. □

Speckle interferometry of Pluto

from David W. Hughes

THIRTY years ago Kuiper used the 200-inch Mount Palomar telescope and a 'disk meter' to measure the diameter of Pluto, the ninth planet of the Solar System. He found a value of about 5,900 km but was considerably worried by possible systematic errors. The disk meter produces an artificial image of variable angular diameter, brightness and colour. These quantities are then adjusted until the artificial image and the image of Pluto look similar. The angular diameter of the planet can then simply be read off. Times change and in March 1978 S. J. Arnold and A. Boksenberg of University College, London and W. L. W. Sargent of the California Institute of Technology used the same telescope and observed the same planet but this time with a speckle interferometer at the Cassegrain focus. The value obtained for the diameter was $3,600 \pm 400$ km, this value however depending on the assumption that the planet disk was limb darkened. Their results have been published in a recent edition of *The Astrophysical Journal* (234, L159; 1979).

Speckle interferometry is a fascinating technique which has been developed extensively in the past 10 years and has been used to measure the diameters of stars, the separation of binaries and to study fine structure on the Sun and on the surfaces of supergiant stars. Atmospheric turbulence distorts the plane radiation wavefront from the astronomical object, a wavefront which has travelled more or less undisturbed through space. This corrugation serves to make a single large telescope mirror act as a multitude of smaller ones with individual sizes of around 10 cm. Small erratic movements are produced in the image position and the overall image is blurred, distorted and considerably enlarged. Turbulence is the main limitation to the resolving power of the telescope and the resulting image can be up to 80 times larger than the diffraction-limited image (the so-called Airy disk).

The speckle interferometric technique relies on taking many short exposure pictures of the object. This freezes the effects of turbulence such that the 'speckles' that make up each individual image are in essence distortion free. Each image is analysed statistically, the end result being a nearly diffraction-limited

image of the original object. Arnold, Boksenberg and Sargent took 170,000 'pictures' of Pluto, each having a 20 millisecond exposure. Each picture was produced by an image photon counting system consisting of a 256×256 element array which could find electronically the centre of each photon event. The information was recorded onto digital magnetic tape. Observations were centred on a wavelength of 5,000 Å with a bandwidth of 350 Å. There were about three photon events in each picture and each picture element was roughly equivalent to an angular extent of 4×10^{-3} arc s on the sky.

The image of Pluto was compared with two stellar images, one being Alpha Herculis A which had a known diameter and the other GC24617 which should be unresolved. The authors found a systematic error of 0.035 ± 0.010 arc s in their results which they thought might be due to an incomplete compensation for atmospheric dispersion and to the possibility that the speckles were not completely stationary over a period of 20 ms.

They conclude that the best fit to the profiles would be obtained if the disk of Pluto subtended an angle of 0.17 ± 0.02 arc s at the telescope and was limb darkened. The presence of limb darkening was deduced by Renschen (*Astronomische Nachrichten* 298, 179; 1977) from observations of Pluto's light variation as a function of time. The authors assume that this limb darkening follows a simple cosine law rather like that of the solar limb darkening. Titan, the 2,440 km radius satellite of Saturn, is also limb darkened like this. Arnold, Boksenberg and Sargent also found marginal evidence for an asymmetry in their data suggesting the presence of Pluto's moon. Also the surface of Pluto seems to have a variable albedo — half of it being covered with high albedo (0.5) methane frost and the remainder being of albedo about 0.13.

Combining the results with other recent observations leads to the conclusion that Pluto has mean opposition visual magnitude of 15.12, Pluto's moon being about 10.8^m. The mass is $(1.9 \pm 0.3) \times 10^{-3}$ Earth masses, the diameter is $3,600 \pm 400$ km and the mean density $0.5 (+0.3, -0.2)$ g cm⁻³. The low value for the mean density suggests that Pluto is made up predominantly of frozen volatiles. The

authors are obviously not too convinced about their limb darkening assumption and they carefully quote another set of results for the non limb darkened case. The mass obviously stays the same but the diameter and density change to 3,000 km and $0.8 (+0.6, -0.3)$ g cm⁻³ respectively.

As time progresses Pluto is looking less and less like a planet and more and more like an escaped planetary satellite. □

Soil seed banks

from Peter D. Moore

As any gardener knows, the soil seems to be full of seeds of a variety of species, some needing only warmth and water to germinate and others whose dormancy is not so easily broken. Harper (*Population Biology of Plants*, Academic Press, 1977) likens these respectively to a current and deposit account in a bank and the term 'seedbank' is one that is frequently used for this latent plant community.

A striking feature of the seed bank is the general disparity between the species contained in it and the present-day vegetation. Many examples could be quoted, such as the work of Kellman (*Can. J. Bot.* 48, 1383; 1970) who found that 70% of the seeds which germinated from soils beneath a century-old *Pseudotsuga* and *Tsuga* forest in British Columbia were *Alnus rubra*, many of the remainder being weeds. The possibility that the seeds had come in from other areas could not be excluded, but it was also considered conceivable that the seed population belonged to an early successional stage following a fire which had taken place a hundred years before.

Besides the disparity between present-day vegetation and seed bank, the seed bank itself can be extremely heterogeneous throughout the site. Van der Valk and Davis (*Can. J. Bot.* 54, 1832; 1976) used a Sorensen similarity index to compare the viable seed flora of mud samples from fresh water marshes in Iowa. Within marshes their indices varied from 2 to 68% and between marshes (eight were studied), indices fell between 24 and 28%. They account for the variability in terms of the time over which any given seed bank has accumulated and the local variation in

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vegetation history.

An exception to the vegetation/seed bank disparity has recently been described by Leck and Graveline (*Amer. J. Bot.* **66**, 1006; 1979) from the freshwater tidal marshes of the Delaware River estuary, New Jersey. These marshes contain a very diverse flora with annuals being particularly important; the tidal fluctuations render the habitat liable to regular disturbance by flooding. Annuals were common in the seed banks of these habitats, accounting for 7 out of the 10 most abundant species, and of the 10 most frequent species germinating, an average of 7.2 species were growing at the collection site.

This exception to the general rule may contain clues to the normal lack of correlation between vegetation and seed banks. The seed bank usually contains a high proportion of early successional (r-selected) species which rely on long-term viability as part of their opportunistic strategy. Late successional (K-selected) species gain no advantage from such persistence but must in consequence re-invasion sites by new propagules following disturbance.

This is an oversimplification of what is evidently a complex of interactions, and a more extensive and systematic analysis has now been formulated by Thomson and Grime (*J. Ecol.* **67**, 893; 1979). They divide seed banks into four types (I-IV) which are thought to differ in their ecological significance. The types are defined by the temporal pattern of germination under normal field conditions. In species of type I and II the seed bank is non-persistent and temporary, seeds germinating either in the autumn after their production, or the following spring. Such types are associated with habitats in which regular gaps are formed seasonally and re-invasion depends on predictable, seasonal factors. In species of type III and IV there exists an increasingly large, persistent seed bank, which confers advantages under conditions where disturbance, and therefore the opportunity for germination, is unpredictable and not closely linked to season. It is natural therefore, that studies of the persistent soil seed banks show a predominance of weed species of essentially type IV. Where successional mature vegetation now dominates such sites, the contemporary dominants are unlikely to be well represented in the soil seed assemblage, for they are likely to possess type I or II features. This will result in the disparity so often observed between vegetation and the local seed flora. The exception, described from the New Jersey tidal marshes, experiences such frequent (but unpredictable) disturbance that the current vegetation is largely composed of type IV seed bank species as of course, is the soil seed bank. The model, developed on the basis of grassland studies, seems robust and will undoubtedly be applied with profit to many other habitats. □

DNA sequence of human papovavirus BK

from Peter M. Howley

THE papovaviruses, including Simian virus 40 (SV40) and its murine counterpart polyoma (Py), are small DNA viruses which have been well studied as models in tumour virology and to elucidate the mechanisms of eukaryotic gene expression. These viruses are oncogenic when injected into hamsters and can malignantly transform some mammalian cells *in vitro*. The first human papovaviruses were isolated in 1971; BK virus from the urine of a renal allograft recipient on immunosuppressive therapy (Gardner *et al. Lancet* **i**, 1253; 1971), and JC virus from the brain of a patient with a rare demyelinating disease, progressive multifocal leukoencephalopathy (Padgett *et al. Lancet* **i**, 1257; 1971). As BK and JC are also oncogenic in newborn hamsters and can transform certain mammalian cells *in vitro*, they have attracted interest because of their potential role in human neoplasia.

Serological studies indicate that BK and JC are ubiquitous in the human population; seroconversion occurs usually in childhood and 70–80% of adults from widespread geographical areas have antibodies specific for each of these viruses. Since BK can be readily propagated in a variety of cultures of human fetal cells and JC grows well only in cultures of primary human fetal spongioblasts (Padgett *et al. Infect. Immun.* **15**, 656; 1977) and to a limited degree in cultures of primary human amnion cells (Takemoto *et al. J. Virol.* **30**, 384; 1979), the molecular studies of the human papovaviruses have been largely limited to BK. Subsequent isolations of BK have been made exclusively from patients undergoing immunosuppressive therapy or suffering from an underlying immunodeficiency. (The laboratory strains of BK currently used are listed in Table 1.) The potential role of BK in human malignancy has been examined by several laboratories

and although conflicting reports have appeared, there is little convincing evidence to associate BK with human cancer (Fiori & DiMayorca *Proc. natn. Acad. Sci. U.S.A.* **73**, 4662; 1976; Israel *et al. Virology* **90**, 187; 1978; Wold *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 454; 1978).

The structure and genetic organisation of the BK genome are similar to those of SV40 and polyoma. The viral genome is a 3.4×10^6 molecular weight double-stranded circular molecule, equally divided into 'early' and 'late' sets of genes (for a recent review of the molecular biology of BK see Howley in *Viral Oncology* (ed. Klein) 489, Raven Press, New York, 1980). The early region, transcribed before the onset of DNA replication, encodes at least two polypeptides, large T antigen (97K) and small t antigen (17K) (Simmons & Martin *Proc. natn. Acad. Sci. U.S.A.* **75**, 1131; 1978). Three structural proteins (VP1, VP2, and VP3) are translated from the late transcripts.

Recently two laboratories have independently determined the complete nucleotide sequence of three different strains of BK: the prototype BK, BK(Dun), and BK(MM) (Seif *et al. Cell* **18**, 963; 1979; Yang & Wu *Science* **206**, 456; 1979). Comparison of these BK DNA sequences with the SV40 DNA sequence (Reddy *et al. Science* **200**, 494; 1978; Fiers *et al. Nature* **273**, 113; 1978) has provided definitive confirmation of earlier DNA-DNA hybridisation studies which indicated extensive sequence homology between the SV40 and BK genomes (Newell *et al. J. Virol.* **25**, 193; 1978; Howley *et al. J. biol. Chem.* **254**, 4876; 1979). The genetic organisation of BK seems identical to that of SV40 and a comparison of the BK viral sequences with analogous regions of the SV40 genome permits the localisation of coding regions and regulatory functions

Table 1 Strains of BK virus

Virus	Source	Reference
BK (prototype)	Urine, renal allograft recipient	Gardner <i>et al. Lancet</i> i , 1253; 1971.
BK(MM)	Urine and brain tumour, Wiskott-Aldrich syndrome	Takemoto <i>et al. J.N.C.I.</i> 53 , 1205; 1974.
BK(Dun)	Urine, Wiskott-Aldrich syndrome	Howley <i>et al. J. Virol.</i> 16 , 959; 1975. Manaker <i>et al. Virology</i> 97 , 112; 1979.
BK(RF)	Urine, renal allograft recipient	Dougherty & Stefano, <i>Proc. Soc. exp. Biol. Med.</i> 146 , 481; 1974.
BK(MG)	Urine, renal allograft recipient	Lecatsas & Prozesky, <i>Arch. Virol.</i> 47 , 393; 1975.
BK(GS)	Urine, renal allograft recipient	Wright <i>et al. J. Virol.</i> 17 , 762; 1976.
BK(DW)	Urine, renal allograft recipient	Wright <i>et al. J. Virol.</i> 17 , 762; 1976.
BK(JM)	Urine, Wiskott-Aldrich syndrome	Howley <i>et al. J. Virol.</i> 16 , 959; 1975.
BK(JL)	Urine, bone marrow allograft recipient	Pauw & Choufoer, <i>Arch. Virol.</i> 57 , 35; 1978.

for both lytic growth and transformation.

There are two regions where differences exist among the three strains of BK sequenced. The first is located in the non-coding tandem repetitive sequences to the late side of the origin of DNA replication. There is a 42 base pair deletion in the BK(Dun) genome compared with the prototype BK sequence. The BK(MM) genome varies more markedly from the other two DNAs in both the sizes and the patterns of these repeated sequences. Tandem repetitive segments are located in the corresponding region of the SV40 genome although they bear no sequence homology to the repeats of BK DNA. Variation also occurs in these tandem repetitive segments among different strains of SV40 and portions of these repetitive segments can be deleted without any apparent effect on the biological function of the virus. Thus there is apparently little sequence constraint in this region among papovaviruses generally or even among strains of the same virus, and its biological function and significance remain obscure.

Differences were also noted in the early region of the genomes of the BK strains. Although the sequences of the early regions of the prototype BK and BK(Dun) are identical, there is a deletion of 262 base pairs in the BK(MM) early region. This deletion removes sequences from the putative intervening sequence for large T antigen, including the likely donor site for the small t antigen splice junction. As a consequence, BK(MM) does not encode a small t antigen (Seif *et al. Cell* **18**, 963; 1979) making it equivalent to some of the constructed early viable deletion mutants of SV40 which map in the analogous region of the SV40 genome. Since BK(MM) can replicate in tissue culture and can induce tumors *in vivo* as well as transform cells *in vitro*, the small t antigen must be dispensable for these functions of BK.

While remarkable similarities can also be found between the genome of BK (or SV40) and that of polyoma, significant differences exist (Soeda *et al. Nature* **283**, 445; 1980). The BK and SV40 genomes contain a large non-coding region between the carboxy terminal ends of large T antigen and VP1 (approximately 100 base pairs), whereas the same region in polyoma contains only seven base pairs. In addition the polyoma genome does not contain the region of tandem repetitive segments in the non-coding region to the late side of the origin of DNA replication as do BK and SV40. The late leader regions of each of the BK and SV40 genomes contain an open reading frame following an initiator AUG referred to as the 'agnogene'. Although proteins encoded by these sequences have not yet been detected, approximately 2/3 of the putative amino acids are conserved

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Testing gravitational theories

from Bruno Bertotti

DURING their mission to Mars in 1976 and 77 the four Viking spacecraft verified Einstein's predictions concerning the additional delay in the propagation of an electromagnetic signal due to the gravitational field of the Sun with an accuracy of 2×10^{-6} , much better than any previous determination. A recent letter to the *Astrophysical Journal* (**234**, L219; 1979) by R.D. Reasenberg and others has now made public the details of this outstanding experimental achievement. This additional delay has the largest value, 250 μ s, when the signal grazes the Sun; with the Viking spacecraft (two orbiters and two landers) it was possible to measure corrections of 50 ns, corresponding to distances of 7.5 m. The main technical reason for this large precision was that the signals came from a lander, firmly anchored to the surface of the planet, whose motion is very regular and predictable.

This is another example of the extraordinary accuracy to which space research has pushed our knowledge of the geometry of the Solar System and, consequently, of such dynamical properties as the planetary masses; and there is still scope for improvement, mainly through the implementation of better telecommunication systems at higher frequencies (including the use of laser signals).

In my view this experiment marks also the end of one phase in gravitational research, which aimed at testing all the

theories of gravitation which are based on the assumption that space-time has a metric structure (described by its curvature) and that the Principle of Equivalence — all local laws have a universal validity — holds. These assumptions have been precisely formulated in a mathematical scheme, which has also led to the classification and the experimental consequences of all possible theories of this kind (see for example, Will, in *Experimental Gravitation* (Ed. Bertotti) Academic Press, New York, 1973). In recent years all these theories have been to a large extent falsified by experiment and theoretical arguments, except general relativity, whose predictions have all been confirmed to a greater and greater accuracy.

It seems, therefore, that the experimental programs within this theoretical framework have passed the point of diminishing returns. This does not mean, however, that in the physics of gravitation there are no open questions and potential anomalies. Perhaps the most important one is the possible change of the constant of gravitation G over a cosmological time scale, which has been extensively studied by P. Dirac and V. Canuto. If this is true, not only is Einstein's theory violated, but the whole of astrophysics and cosmology must undergo a drastic revolution. The tests of the constancy of G are not yet sufficiently precise to exclude this possibility; and precise geometrical measurements in the Solar System, especially if extended over a long period of time, will probably be crucial.

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between the BK and SV40 genome suggesting perhaps, that a protein exists. No corresponding 'agnogene' is present in the late leader region of polyoma.

The most significant difference between the DNAs of BK (or SV40) and Py lies in the genetic organisation of the early regions and possibly reflects the different host ranges of these viruses. The BK and SV40 early regions are extensively homologous throughout and each encodes only two known major early proteins, large T and small t antigens. The Py early region encodes a third early protein, middle T antigen (Ito *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 4666; 1977; Hunter *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 5917; 1978). Whereas the amino acid sequence of the Py small t antigen is remarkably similar to those of BK and SV40, the predicted Py large T antigen differs in two regions significantly from those of BK and SV40. First there is a 130 amino acid segment ('insertion') unique to the Py large T antigen located 100 nucleotides after the

early acceptor splice junction which has no counterpart in BK or SV40. The second difference is the carboxy terminus of the BK and SV40 large T antigens which is essentially absent from the Py large T. Interestingly the segment of DNA encoding this unique carboxy terminal portion in the BK and SV40 genomes is open in a second reading frame for approximately 300 nucleotides providing an additional potential coding segment. Putative peptides encoded by this segment of BK and SV40 would be hydrophobic and might thereby be used in associating a protein with a membrane; however, such peptides have not yet been demonstrated.

100 years ago

The following arrangements have been made at the Royal Institution for the lectures after Easter. Tuesdays: — Prof. Huxley — Two Lectures on Dogs, and the Problems connected with them; Mr. Robert H. Scott, F.R.S. — Four Lectures on Wind and Weather.

From *Nature* **21**, 11 March, 451; 1880.

More light on membrane protein mobility

from Michael T. Flanagan

ONE of the most useful and unambiguous techniques for measuring the mobility of proteins in a membrane is fluorescence recovery after photobleaching (FRAP). In this technique, a cell is labelled as specifically as possible by saturating a membrane-localised receptor with its ligand which has previously been labelled with a fluorescent dye. As described in the original papers, a small circular spot ($\sim 8 \mu\text{m}$ in diameter) is bleached on the surface of the cell by exposing it to a focused laser beam of the appropriate wavelength to be absorbed by and destroy the fluorescent dye. The appearance of fluorescence within the circle after bleaching can arise only from the diffusion of unbleached material into the spot. The rate of this recovery is related in a relatively simple manner to the diffusion constant of the labelled molecular complex (Edidin *et al. Science* **191**, 466; 1976; Schlessinger *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 2409; 1976). The essentials of the method were first described by Poo and Cone (*Nature* **247**, 438; 1974) who, in the absence of laser technology, measured the diffusion constant of rhodopsin in amphibian disk membranes using the easily bleached natural chromophore, retinal. They found a value approximately one order of magnitude less than that found, by resonance techniques, to characterise the lipids of natural membranes. The difference between protein and lipid mobility has emerged as the most interesting theme linking the various FRAP experiments.

A difference might be expected simply because the proteins are much larger than the lipids. However, the first FRAP experiments carried out on mitogens and antibodies bound to cell membrane receptors, for example, concanavalin A on myoblasts, IgE on mast cells, demonstrated that the mobilities of these protein receptor complexes were determined by more than their size. In general two populations of complex were found; one immobile and one with a diffusion constant up to two orders of magnitude lower than that of the membrane lipid. The diffusion constant of the latter population fell dramatically in the presence of cytochalasin B. This microbial metabolite does not alter the mobility of the membrane lipids. As it is known to combine with elements of the cytoskeleton these FRAP results strengthened the growing belief that there is a physical link between some membrane receptors and the cytoskeletal structure underlying the membrane. FRAP has also been used to study lipid mobility in its own right and such studies have given additional

confidence in the validity of the technique. The diffusion constants obtained using small fluorescent lipid probes confirmed the values obtained from magnetic resonance studies (Wu *et al. Biochemistry* **16**, 3939; 1977). This study also illustrated the limitations of the technique. Diffusion constants in liquid crystalline phases of pure lipids, for example, in dimyristoylphosphatidylcholine above 23°C , could be measured but not those in the gel phase, that lipid below 23°C . To extend the detection limit of the technique to cover these high viscosity membranes Smith and McConnell (*Proc. natn. Acad. Sci. U.S.A.* **75**, 2759; 1979) introduced a very elegant modification to the technique that has, in addition, assumed considerable importance in examining possible interactions between receptors and cytoskeleton.

Instead of bleaching a spot Smith and McConnell bleached a series of parallel lines by first passing the laser beam through a ruled grating. A square wave pattern is produced which rapidly forms a sine wave pattern. The diffusion constant is obtained from the decay constant of the amplitude of the sine wave. Not only is the sensitivity improved but gross cellular movements can be compensated for as the frequency of the discrete pattern is not altered by protein-receptor complex or labelled lipid diffusion. McConnell's group have now examined the mobility of the M13 phage coat protein incorporated into an artificial membrane at temperatures both above and below the phase transition temperature of the lipid. The results in the gel phase were compatible with either protein aggregation or phase separation into protein-rich and protein-poor domains. In the liquid-crystalline phase a diffusion constant for the protein comparable with that of the lipid was found (*Biochemistry* **18**, 2256; 1979).

In their next modification to the FRAP method McConnell's group returned to the problem of protein mobility in natural membranes. They have shown that it is possible to measure the anisotropy of diffusion in a membrane by bleaching a two dimensional periodic grid pattern (*Proc. natn. Acad. Sci. U.S.A.* **76**, 5641; 1979). The reduction of the data is no longer simple and requires Fourier image analysis. By aligning one axis of the bleached grid pattern with the predominant direction of the 'stress' fibres of mouse embryo fibroblasts showing anisotropic arrangements in their cytoskeletal components they were able to obtain the diffusion constants of the succinylated concanavalin A receptor complex in the directions both parallel and perpendicular to the 'stress' fibres. These differed by up to a factor of 10; the parallel motion being the faster. The presence of a physical link between this complex and the

cytoskeleton now seems certain. Whether it is a direct anchoring link or a more subtle one involving exclusion of the complex from certain regions of the membrane cannot be determined by this technique alone but FRAP clearly will be a powerful tool in the study of the fate of such complexes.

The factors that determine protein mobility in a complex membrane, even in the absence of cytoskeletal interactions, are not fully understood. This is in part because the structure and dynamics of the mixed lipid membranes themselves are not totally clear. But it is also impossible to predict with certainty how a protein will behave in a hypothetical continuous homogeneous two-dimensional fluid. The equations describing diffusion in three dimensions possess neat analytical solutions; when constrained to two dimensions they do not.

Saffman and Delbrück (*Proc. natn. Acad. Sci. U.S.A.* **72**, 3111; 1975) have examined the consequences of this paradox in the context of protein diffusion in a biological membrane. The availability of methods for the accurate determination of both lateral and rotational diffusion constants (correlation spectroscopy for the latter) should now allow the theoretical model derived by these authors to be tested and several of the groups using the FRAP technique see this as one of their goals. Experiments are also being devised to examine the effect of the multicomponent nature of the lipid membrane on protein diffusion. Vaz *et al.* (*Proc. natn. Acad. Sci. U.S.A.* **76**, 5645; 1979) have taken the apolipoprotein, ApoC-III, as a model of a membrane protein and examined the effect of adding cholesterol to phosphatidylcholine bilayers on the mobility of the protein when bound to such bilayers. They believe that the protein is bound in the head group region of the membrane and that its mobility represents the upper bound for those values that will be found to characterise the integral membrane proteins.

Given the advanced state of the art of membrane reconstitution FRAP studies of several integral proteins are now feasible. In one of the first of these Schindler, Osborn and Koppel (*Nature* **283**, 346; 1980) measured the diffusion constants of the phospholipid, lipopolysaccharide and matrix protein in a reconstituted *Escherichia coli* outer membrane. They cannot reconcile their results with the model of Saffman and Delbrück and suggest that macromolecular diffusion in a membrane is more analogous to non-electrolyte diffusion through a polymer network than through a homogeneous fluid. FRAP experiments carried out on reconstituted systems containing enzymes whose activity has been shown to depend on the state or nature of the surrounding lipid, for example the ATP-dependent Ca^{2+} -transporting protein of the sarcoplasmic reticulum, will be of especial interest. □

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REVIEW ARTICLE

Highly excited atoms

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Highly excited atoms are oversize, fragile species currently studied for both intrinsic interest and as detectors of IR and microwave radiation. Traditional quantum mechanics provides accurate descriptions of atoms in these exotic states and modern dye lasers are responsible for the new capability in their spectroscopy.

DURING the past four or five years the spectroscopy of very highly excited states of atoms and molecules has advanced dramatically. These atoms are especially interesting because the active electron is much less perturbed by the other electrons in the atom than when it is in lower states, and the atom is much closer to the ideal hydrogen-like model so frequently used. Such highly excited atoms are very rare in nature because of their large size and fragility, but they are found in interstellar space (H I regions). The primary reason for the increase in the laboratory study of atoms in these exotic configurations is the recent availability of tuneable dye lasers which can provide beams of very high spectral density over the entire optical spectrum^{1,2}. These laser beams can be easily directed into high vacuum regions where atoms are relatively free of perturbing interactions, and can be tuned to excite the atoms to specific highly excited levels of interest.

Although series of atomic levels have been studied for nearly 100 years (Balmer described the regularity of the visible lines of hydrogen in 1884), most spectroscopy experiments have been restricted to the few lowest lying levels, particularly those accessible by electric dipole transitions from the ground state. Tuneable lasers allow direct excitation to high levels, permit stepwise excitation by two sequential electric dipole transitions to states not directly accessible from the ground state, and can induce second-order processes such as two quantum excitation to otherwise inaccessible levels. With proper choice of laser frequency, intensity, and suitable applied external fields, one may selectively populate almost any excited atomic state. Atoms in highly excited states are called Rydberg atoms.

In the following description of Rydberg atoms we shall use atomic units, (AU). Energy is measured in units of twice the Rydberg constant ($\cong 2.2 \times 10^{-5} \text{ cm}^{-1}$), charge and mass in units of the electron's charge and mass ($e = 1$ and $m = 1$), and time in units such that \hbar also equals unity. Table 1 shows some common quantities measured in these convenient and ubiquitous units.

Table 1 Atomic units

$e = \hbar = m = 1 = \alpha c$
Unit of distance = $a_0 = \hbar^2/me^2 = 5.3 \times 10^{-9} \text{ cm}$
Unit of velocity = $ac = e^2/\hbar = 2.2 \times 10^8 \text{ cm s}^{-1}$
Unit of time = $\hbar^3/me^4 = 2.4 \times 10^{-17} \text{ s}$
Unit of energy = $2R = mc^2\alpha^2 = 2.2 \times 10^5 \text{ cm}^{-1} \times hc$
Unit of E field = $e/a_0^2 = 5.1 \times 10^9 \text{ V cm}^{-1}$
Unit of B field = $c/\alpha = 2 \times 10^9 \text{ G}$

Atomic structure, Bohr model

Many properties of Rydberg atoms can be understood correctly in terms of the relatively simple notions used to describe hydrogen atoms. For example, the deBroglie hypothesis that an integral number of electron waves must fit on the circumference of a classical orbit gives the same formula for the energy levels derived many years earlier by Bohr, and this formula gives a very

good approximation to the energy levels of Rydberg atoms. We have $E_n = -(mc^2/2)(\alpha Z/n)^2$ which becomes

$$E_n = -1/2n^2 \text{ AU} \quad (1)$$

for $Z = 1$. The energies are negative and approach each other and zero at larger values of n , the principal quantum number. The energy separation between adjacent levels is approximately $\Delta E = 1/n^3$ and Fig. 1 shows the Bohr energy levels given by this formula. (Contrast these energies with the energies $E_n \cong E_0 n^2$ of a particle bound in a square well, where the energy levels spread further apart with increasing n and there are a finite number of bound states.)

In the Bohr model, the speed of the electron is $\alpha c/n$ which becomes $1/n \text{ AU}$, and the radius of the orbit is $(n\hbar)^2/me^2$ which becomes just $r = n^2 \text{ AU}$. The picture that emerges of a Rydberg atom is one of an ion core and an isolated electron very far away, floating lazily around in a slow orbit, much like a distant planet of the Solar System. For $n = 100$ the orbit is as large as a living cell and the electron speed is only $2 \times 10^4 \text{ m s}^{-1}$.

Although the Bohr energy formula gives very good values for the hydrogen atom, it does not do as well as for other atoms because the valence electron's orbit may take it inside the closed-shell core where the potential descends very much more steeply than that of hydrogen. The electron therefore has much lower energy as shown by the s-levels in the central part of Fig. 1. The low angular momentum states (penetrating orbits) of alkali metals are therefore much more tightly bound than either the high angular momentum states (non-penetrating orbits) which see a nearly Coulombic potential or the corresponding n states of hydrogen. The differences between the high angular momentum states of alkalis and those of hydrogen arise because the ion core is composed of mobile electrons whose orbits can be perturbed by the valence electron. In the language of classical electromagnetic theory, we say that the core is polarisable and that its polarisability influences the energy of the high angular momentum states³.

Although departure of alkali energy levels from those of hydrogen arises from two rather distinct mechanisms depending on the angular momentum of the alkali state, the energy levels E_n of all the alkali levels are given very accurately by a single, empirically determined, minor modification to the Bohr formula:

$$E_n = -\frac{1}{2(n-\delta_l)^2} \cong -\frac{1}{2n^2} \quad (2)$$

Here δ_l is the quantum defect for a particular angular momentum l and a particular atom, and is nearly independent of n . Some representative values are given in Table 2. We expand equation (2) for the case $n \gg \delta$ and find

$$E_n \cong -\frac{1}{2n^2} - \frac{\delta}{n^3} = -\frac{1}{2n^2} \left(1 + \frac{2\delta}{n}\right) \quad (3)$$

for the energies of the alkali energy levels which agrees very well with spectroscopic data.

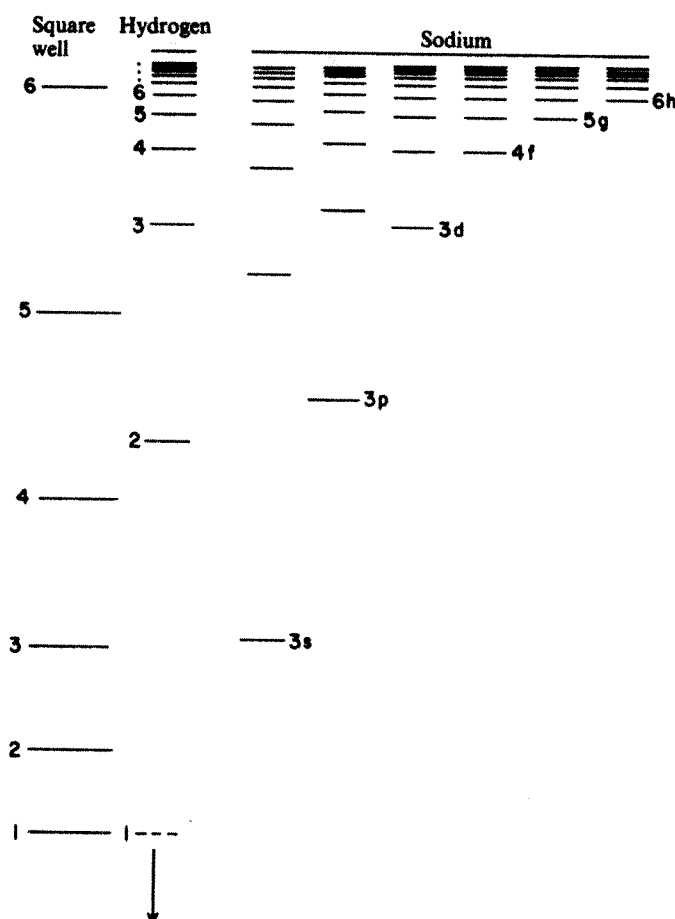


Fig. 1 Energy levels for various quantum mechanical systems. The left edge is a square well, the centre is the hydrogen atom, and the right half is the sodium atom.

Quantum mechanical discussion —no external fields

The quantum mechanical description of alkali atoms in Rydberg states begins with the Schrödinger equation for one electron in a Coulomb field, treating other effects such as spin orbit interaction (which decreases as $1/n^3$) as perturbations. The Schrödinger equation for hydrogen separates into a radial and angular part and the total wave function is the product $R_{nl}(r)Y_{lm}(\theta, \phi)$. The radial functions $R_{nl}(r)$ are the product of a Laguerre polynomial and a decreasing exponential in r . There are $(2l+1)Y_{lm}$ s that are solutions for each value of $l < n$ and that all have the same energy. Relativistic and radiative corrections produce the usual fine structure of these energy levels (spin-orbit splitting, Lamb shift, and so on) lifting the degeneracy among the states of different l and J . On the other hand, the effects of core polarisability and core penetration produces an electrostatic fine structure which also shifts the energy of states of different l , and these shifts are usually much larger than the relativistic ones. In fact, the spin-orbit splitting and Lamb shift are usually neglected in Rydberg spectroscopy because they are much smaller than other energy shifts (such as those arising from stray electric fields).

The quantum mechanical treatment^{3,4} of the perturbations on the valence electron in a high angular momentum state of an alkali atom begins with a derivation of the Hamiltonian term which arises from its interaction with the core. The electron polarises the core and the dipole thus induced acts back on the electron. An electron located at $r \gg r_{\text{core}}$ produces an electric field proportional to $1/r^2$ at the core, and the induced dipole moment produces an electric field at r proportional to $1/r^3$. Therefore, the additional field seen by the electron varies as $1/r^5$ and the potential of that field is proportional to $1/r^4$. We write

$$V_{\text{dipole}} = \frac{\beta}{2r^4} \quad (4)$$

where β (AU) is the classical polarisability and is of the order of unity (see Table 2).

We consider this potential as a perturbation to the Coulomb potential, evaluate its expectation values using the unperturbed wave functions of the hydrogen atom in the spirit of first-order perturbation theory (diagonal terms only), and find an energy shift

$$\begin{aligned} \Delta E_{\text{dipole}} &= \langle R_{nl}(r) | \frac{\beta}{2r^4} | R_{nl}(r) \rangle \\ &= \frac{\beta(3n^2 - l(l+1))}{4n^5(l+3/2)(l+1)(l+1/2)l(l-1/2)} \end{aligned} \quad (5)$$

For $l \sim n \gg 1$, $\Delta E_{\text{dipole}} \sim 1/n^8$ which is an extremely small correction to the energies. On the other hand, for fixed values of $l \ll n$ this term varies approximately as $1/n^3$, and the approximation is really very good. This is precisely the energy dependence needed to make the quantum defect formula equation (3) work, and shows why that formula can be very accurate for energies associated with induced dipole moments of the core. Of course, equation (5) is not valid for $l=0$, but the energy shifts for s states (penetrating orbits) are dominated by the increased Coulomb energy and not by core polarisability.

The energy arising from the induced dipole moment is the largest contributor to the shift of alkali energies away from the hydrogen energies (for non-penetrating orbits), but there are other sources of energy shifts. For example, the electric field at the core from the valence electron is not uniform (it depends on $1/r^2$) and the response of the core to either a uniform or non-uniform field may not be linear. Therefore the induced charge distribution need not correspond to a pure dipole moment, but may have quadrupole, octopole, and higher order terms. For each of these there is an addition to the Hamiltonian which can be treated similarly to the dipole term. It is a very curious property of the hydrogen radial wave functions that, for all potentials, V , that vary as an inverse integer power of $r > 1$, the leading term for the energy shift is proportional to $1/n^3$ (ref. 5). The result is that the quantum defect formula of equation (3) provides a very accurate prediction for the energy levels from interactions of different types (penetration as well as polarisation) because multipole terms are all proportional to some inverse powers of r . Finally, neither the electron nor the core is at rest resulting in various dynamic contributions to the polarisability (see discussion in ref. 4).

Equations (3) and (5) can be used to find approximate values for the quantum defects of non-penetrating states:

$$\delta_l \approx 3\beta/4l^5 \quad (6)$$

and the $1/l^5$ dependence is consistent with spectroscopic data as exhibited by the entries in Table 2. We can therefore use the tables of spectral data to determine the dipole polarisabilities of the ion cores. The deviations from a perfect fit to equation (6) (or the unapproximated results of equation (5)) can be used to determine the quadrupole polarisability or other higher order effects (such as dynamic effects, and retardation). Furthermore,

Table 2 The core polarisabilities and quantum defects for several of the alkalis*

	Core polarisability (AU)	Quantum defect			
		s $l=0$	p 1	d 2	f 3
Lithium	0.19	0.4	0.05	0.002	—
Sodium	1.0	1.35	0.85	0.014	0.0015
Potassium	5.5	2.18	1.71	0.27	0.010
Rubidium	9.0	3.14	2.65	1.34	0.016
Caesium	14	4.06	3.58	2.47	0.033

*For more detailed information see ref. 9.

high resolution studies of Rydberg atoms with particularly simple cores (helium, lithium) can provide a basis for comparison between atomic theory and experiment.

Rydberg atoms in external fields

The description of Rydberg atoms in weak external fields is very similar to that of ground state atoms, but the field strengths that can be called weak are very restricted. As highly excited electrons are weakly bound, moderate external fields can have large effects on them.

Consider first a semi-classical description of the effects of a magnetic field. For very small fields the atoms exhibit the usual Zeeman effect. But larger fields induce considerable diamagnetism in Rydberg atoms as a result of their large size: their orbital magnetic moments $M = ia$ (a = area) scale as $(1/n)$ ($n^4 = n^3$ and the interaction is quite large).

A complete quantum mechanical discussion of diamagnetism in Rydberg atoms is beyond the scope of this review and some unsolved problems are now attracting considerable attention. An outline of some first steps will be presented here. Note that the Hamiltonian for an electron in a field is (in AU)

$$\mathcal{H} = \frac{1}{2}(\mathbf{p} - \mathbf{A})^2 + V \quad (7)$$

where \mathbf{A} is the vector potential and V is the scalar potential of the electrostatic binding field. For $\mathbf{A} = 0$ the equation is separable; for a uniform magnetic field \mathbf{B} , $\mathbf{A} = \frac{1}{2}\mathbf{B} \times \mathbf{R}$. The $\mathbf{A} = 0$ solutions in spherical coordinates are also eigenfunctions of $\mathbf{A} \cdot \mathbf{p}$, the cross terms in the square in equation (7), and give the usual Zeeman effect. Although the A^2 term is negligibly small in ground state atoms, its r^2 (or n^4) dependence makes it significant for Rydberg atoms. When it cannot be neglected, the Hamiltonian is not separable in any coordinate system and a perturbation treatment is necessary.

We choose the basis set found from the solution of the separable Hamiltonian and evaluate the corrections to the energies from the Hamiltonian matrix elements. The first order diamagnetic shift⁶, found from equation (7), is

$$\Delta E_{\text{dm}}^{(1)} = \langle \psi | \mathcal{H}_{\text{dm}} | \psi \rangle = \langle \psi | (\alpha^2/8) B^2 r^2 \sin^2 \theta | \psi \rangle \quad (8)$$

where r and θ are the usual spherical coordinates and $\psi = R_{nl}(r)Y_{lm}(\theta, \phi)$ is the solution to the separated Schrödinger equation. To evaluate the angular part of this expression we use $\sin^2 \theta = (\text{constant}) Y_{2+2} e^{+2i\phi}$ and the properties of integrals of products of spherical harmonics. The radial part can be calculated from the Laguerre polynomials and the final result is

$$\Delta E_{\text{dm}}^{(1)} = \frac{\alpha^2 B^2}{8} \frac{n^2 [5n^2 + 1 - 3l(l+1)] (l^2 + l - 1 + m_l^2)}{(2l-1)(2l+3)} \text{ AU} \quad (9)$$

For $n \gg l \sim 1$, this becomes ($m_l = 0$)

$$\Delta E_{\text{dm}}^{(1)} \cong \frac{\alpha^2 B^2}{8} n^4 \text{ AU} = 5 \times 10^{-7} B^2 n^4 \text{ cm}^{-1} (B \text{ in Tesla}) \quad (10)$$

Observe that, for $n \approx 32$, the diamagnetic energy is comparable to the Zeeman energy (typically 1.4 MHz G^{-1}) at $B = 2 \times 10^{-5} \text{ AU} = 1 \text{ T}$. Also, the ratio of the diamagnetic shift to the Coulomb energy interval scales as n^7 so that these become comparable for $n \approx 42$ in the same field.

More accurate calculations of diamagnetic energy shifts require the off-diagonal matrix elements of the A^2 part of the Hamiltonian. It is clear from the $\sin^2 \theta$ dependence that the only non-vanishing angular integrals are those for which $\Delta l = \pm 2, 0$: A^2 can only connect states of the same parity and must satisfy a 'triangle condition' on the l values. It is also clear that $\Delta m = 0$ and that there is no selection rule on n . For strong fields and/or large values of n , perturbative calculations are not satisfactory and other methods of calculations are being explored.

Next we consider a semi-classical description of the effects of an electric field. Note that, even for very weak fields, there are

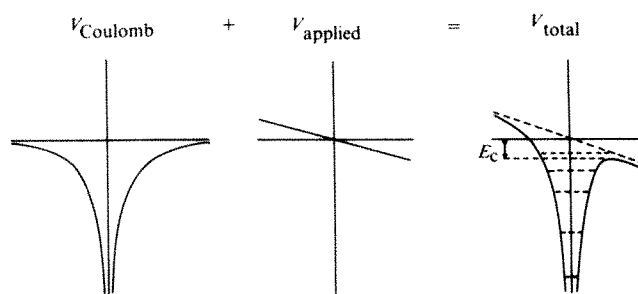


Fig. 2 The potential at the right results from adding the potential of a uniform field to that of the Coulomb field. E_c is indicated as the point where the electron is no longer bound to the force centre.

no perfectly bound states because there is always a finite distance from the atom where the electrical potential will be lower than the electron's binding energy. We therefore expect all expansions in powers of the field to diverge eventually. As an electric field acts on an atom through its induced dipole moment (which scales as r or n^2), and because the interaction is proportional to r , we expect energy shifts that scale as r^2 or n^4 and that can become quite significant in Rydberg atoms.

We begin a quantum mechanical description of Rydberg atoms in electric fields by noting that the Schrödinger equation can be separated in parabolic coordinates as well as the more common spherical coordinates even in the presence of a z -directed electric field⁷.

In contrast to the weak magnetic field case where the zero field eigenfunctions are also eigenfunctions of $\mathbf{p} \cdot \mathbf{A}$, the resulting radial differential equation for an electric field is not exactly soluble. Nevertheless, having the angular part separated makes the energy and eigenfunction calculations much easier. Parabolic coordinates are defined by

$$\begin{aligned} \zeta &= r + z = r(1 + \cos \theta) \\ \eta &= r - z = r(1 - \cos \theta) \\ \phi &= \phi \end{aligned} \quad (11)$$

We can write the solutions to the zero field Schrödinger equation as

$$u(\zeta, \eta, \phi) = u_1(\zeta)u_2(\eta)\Phi(\phi) \quad (12a)$$

where $\Phi(\phi) = e^{im\phi}$ as before and the energies of the levels are

$$E_n = -\frac{1}{2n^2} \text{ AU}, \quad n = n_1 + n_2 + |m| + 1 \quad (12b)$$

where n_1 and n_2 are the quantum numbers associated with the solutions for $u_1(\zeta)$ and $u_2(\eta)$.

In the presence of an electric field F the perturbation Hamiltonian is Fz (in AU) and we note that the matrix of Fz is diagonal in m because z commutes with L_z . In general the first-order perturbation correction to the energies is zero because Fz is an odd operator and has no diagonal matrix elements. However, hydrogen has some degeneracies between states of opposite parity which result in eigenfunctions of no definite parity, thereby producing finite diagonal elements. The first-order energy shift is then

$$\Delta E_s^{(1)} = \langle \psi | Fz | \psi \rangle = \frac{3}{2} \frac{Fn}{Z} (n_1 - n_2) \quad (13)$$

which scales as n^2 . In the more general case where there is no degeneracy, one must use the off-diagonal matrix elements and

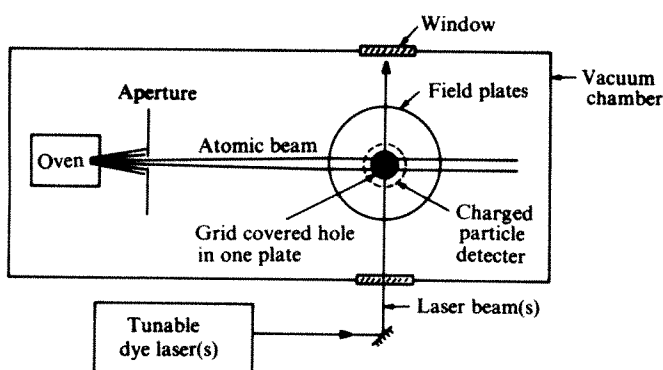


Fig. 3 A typical apparatus showing the major components.

second-order perturbation theory to find

$$\Delta E_s^{(2)} = -\frac{F^2}{16} \left(\frac{n}{Z}\right)^4 [17n^2 - 3(n_1 - n_2)^2 - 9m^2 + 19] \quad (14)$$

which scales as n^6 . The ratio of the second order Stark shift to the Coulomb energy intervals scales as n^9 and becomes unity for $F = 10^{-6} \text{ AU} = 5 \text{ kV cm}^{-1}$ at $n = 21$.

The Stark structure of the alkali Rydbergs is still being actively investigated. One can do higher order perturbation theory⁸, diagonalisation with a truncated basis set⁹, or other approximate calculations⁷.

Because of their relatively small binding energy, Rydberg atoms can be readily ionised by electric fields. We write the potential for a Rydberg electron as $V = -1/|r| - Ez$ (see Fig. 2) and find that it has a maximum value $V_{\text{max}} = -2\sqrt{F}$ at $z = 1/\sqrt{F}$. An electron with energy $-1/2n^2 > V_{\text{max}}$ can escape. The condition for field ionisation is thus $F \geq 1/(16n^4)$ which occurs at $F = 10^{-6} \text{ AU} = 5 \text{ kV cm}^{-1}$ for $n = 15$.

Of course, field ionisation is not quite so simple. First, some electrons occupy states with wave functions concentrated away from the saddle-point produced in the potential by the electric field. They may have enough energy to field ionise, but do not sample the potential at the right place. Also, electrons with insufficient energy to ionise classically may escape by tunneling under the barrier. Further complications arise from the mixing of the wave functions by the field resulting in excited states that are superpositions of zero-field eigenfunctions. Field ionisation is a separate topic of serious investigation¹⁰.

Experimental description

The apparatus needed for the experimental study of Rydberg atoms is modest by comparison with other experimental facilities¹¹. One begins with a simple beam apparatus, consisting of a small stainless steel oven that can be heated to a few hundred degrees, contained in a vacuum system of characteristic linear dimension 0.5 m. Although some metals require a heat pipe oven, and some Rydberg spectroscopy has been done on rare gases which require no oven, the system described above is the most common. The atomic beam is collimated by a series of apertures and crossed by one or more laser beams which enter the vacuum system through appropriately placed windows (see Fig. 3).

At first it might seem that detection of Rydberg atoms could be very much the same as detection of other excited atoms: one simply looks at the radiation emitted when the atom decays to its ground state. More careful consideration reveals that Rydberg atoms do not decay very well by radiation, and in fact may have lifetimes long enough for the atoms to crash into the wall at the far end of the apparatus without ever emitting light. Transitions down to adjacent Rydberg levels are very weak and slow

because their frequencies are very low ($\Delta E = 1/n^3$) and spontaneous transition probabilities are proportional to the cube of the frequency ($1/n^9$). Transitions down to low lying or ground states, which have much higher frequencies, are very weak because the radial wave functions of Rydberg levels have very many oscillations and the integral of such oscillatory functions multiplied by one with only very few nodes is a small number, nearly zero. The result is that detection of Rydberg atoms by spontaneous emitted radiation is ineffective.

The usual procedure is to mount flat plate electrodes on either side of the atomic beam and apply a strong, d.c. electric field to the atoms. As the Rydberg electron is so far away from the nucleus and core, it is bound by a Coulomb field that is quite weak and it can easily be pulled away by the applied field and detected. Field ionisation, as described earlier, is very efficient for Rydberg atom detection.

Figure 3 shows a typical apparatus. The tuneable lasers are pumped by powerful beams from nitrogen or Nd:YAG lasers. The lasers are tuned to the transitions of interest and directed into the apparatus where they cross the atomic beam between the field plates. Time delay circuits triggered by the laser pulse apply the ionising field to the Rydberg atoms sometime after they have been created, and the detector measures the number of ions or electrons produced.

Applications of Rydberg spectroscopy

The signals are typically studied as a function of laser frequency, applied d.c. electric⁹ or magnetic fields^{15,16}, applied r.f. or microwave fields¹²⁻¹⁴ (which induce transitions between fine structure levels or to other Rydberg levels), background gas pressure¹⁷, or combinations of these and other influences. Figure 4 shows signals taken as a function of laser frequency at different values of applied electric fields (much smaller than the ionising field)¹⁸. Each vertical line is a wavelength scan taken at a different value of the applied electric field. The Stark spectrum of the sodium atom is dramatically laid out in Fig. 4.

The new knowledge which has come and will continue to develop from the study of Rydberg atoms spans many areas of atomic physics. For example, Stark shifts are greatly enhanced in highly excited states because the electron wave function is spread out over such a large region. The n^6 dependence described by equation (14) predicts a quadratic Stark shift of about $2 \times 10^{-3} \text{ AU} = 405 \text{ cm}^{-1}$ for an $n = 35$ atom in a field of $10^{-6} \text{ AU} = 5 \text{ kV cm}^{-1}$. As modern pulsed dye lasers can easily resolve 0.1 cm^{-1} (continuous wave (c.w.) dye lasers can resolve $3 \times 10^{-5} \text{ cm}^{-1} = 1 \text{ MHz}$), the Stark shifts arising from fields as small as 80 V cm^{-1} are quite noticeable in pulsed laser spectra, and from stray fields as small as 1.5 V cm^{-1} in c.w. (or r.f.) spectra. The Stark spectra of Rydberg states of several alkalis have been recently studied and described in detail¹⁸.

The Zeeman effect has no such spectacular enhancement. The magnetic shift of atomic energy levels is proportional to the Lande g -factor which depends only on the spin and the angular part of the wave function $Y_{lm}(\theta, \phi)$, and varies by no more than a factor of three over almost all atomic states. However, we have seen that the diamagnetic term or quadratic Zeeman term becomes important because of the large area (proportional to n^4) of the electron orbit. Diamagnetic effects of atoms in low lying states can only be observed in extraordinarily large magnetic fields, but for Rydberg atoms the effects are easily measured in laboratory fields^{15,16}.

When atoms are subjected to extremely strong electric or magnetic fields, that is, those fields which produce forces comparable to the Coulomb binding force, their structure is markedly changed. When the external field is so large that it can no longer be considered a perturbation, the ordinary solutions to the Schrödinger equation are not even approximately right. Theoretical studies of atoms in such conditions have been

stimulated by interest in plasma and astrophysical problems, but experimental measurements have not been done because laboratory fields can not be made large enough¹⁹. Rydberg atoms can provide a testing ground for the theories of atoms in strong fields because laboratory fields can easily be made to satisfy the strong field condition. We have already seen that field ionisation, that is, the process of atoms being torn apart by an external field, is a routine phenomenon in Rydberg studies. Further experiments should provide insights into the behaviour of matter at the surface of neutron stars (magnetic fields $\approx 10^{12}$ G) and in hot plasmas (very strong electric fields).

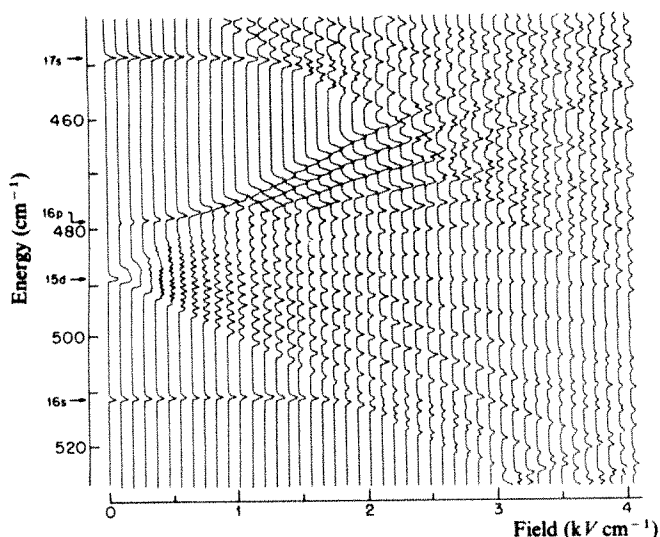


Fig. 4 The Stark spectrum of sodium (taken from ref. 18).

Spontaneous radiative transitions between Rydberg levels are very slow because the rate is proportional to the cube of the frequency, but stimulated transitions can be fast because the rate depends only on the strength of the radiation and the transition moment (square of the dipole matrix element) between the two levels involved. As the transition moment between two Rydberg states is very large (proportional to n^4), the rate is also very large and minute amounts of power are required to induce transitions. It is, therefore, fairly easy to do r.f. or microwave spectroscopy on these atoms^{12,13}. In fact, the matrix elements are so large that power broadening is difficult to avoid, and higher order transition processes (two or more quanta) occur readily.

There are many different kinds of r.f. experiments to be done. For example, r.f. spectroscopy of Rydberg atoms can produce high precision measurements of atomic parameters because of the very narrow linewidths (long lifetimes) of the transitions²⁰. One might measure fine structure splittings¹⁴, quantum defects, hyperfine interactions, or even fundamental constants by careful experiments on Rydberg atoms. Also, even in a moderately strong r.f. field, very high order processes (multi-photon) are easily observable²¹. Not only can these be used for spectroscopic investigations, but also for the testing of theories of ground state atoms in very strong optical frequency fields (high intensity laser light).

As transitions between Rydberg states can be induced by very tiny amounts of radiation, one must consider all possible sources in the description of such experiments. Although the power density of black-body radiation at room temperature is extremely small and is normally only considered in low temperature experiments, careful calculations and observations have shown that this radiation can appreciably shorten the lifetime of highly excited states by stimulated transitions²². One also expects that cooperative effects such as super-radiance and maser action may also be possible in a sufficiently dense population of Rydberg atoms.

The enormous transition moments of Rydberg atoms can be exploited to make sensitive detectors of low frequency radiation²⁰. As transitions can be detected by selective field ionisation, each quantum absorbed from an IR or microwave field produces a charged particle which can be readily detected. The result is a high efficiency, low noise, tuneable (via Stark effect), quantum detector of great interest in low temperature studies, astrophysics, communications, and so on.

A beam of atoms in Rydberg states can be thought of as transporting very low energy electrons at nearly uniform speeds. It is very difficult to make an electron beam monochromatic because of the thermal energy spread of the source, but Rydberg atoms divide the thermal energy between the electron and the very heavy nucleus so that the velocity distribution is very narrow. Collisions can be made either with Rydberg atoms directly on the target or by gently photoionising the highly excited electron and using it alone on the target. The resolution of electron collision studies on gases, beams and surfaces will be greatly enhanced by the use of Rydberg atom electron sources.

The previous discussion above has been restricted to Rydberg states of alkali metal atoms. It is possible to study the Rydberg states of molecules²³ or atoms with two or more valence electrons²⁴ (such as alkaline earths) and the added richness of the spectra provides new information. For example, after one electron is promoted to a Rydberg state, a second electron can be raised to one of the ion core's excited states. The atom now has too much internal energy to remain bound and autoionises, emitting an 'Auger electron'. This process may be enhanced by configuration interaction with another nearby state and precise studies can be made. A relatively new theoretical structure called multichannel quantum defect theory (MQDT) has been developed to describe these processes²⁵ which can be measured carefully and cleanly by Rydberg spectroscopy.

Conclusion

This discussion of the physics derived from Rydberg spectroscopy cannot be complete because of both the breadth of the field and its rate of development. There are a great many other topics which could not be included here because of space limitations. We have seen that many of the properties of Rydberg atoms can be accurately described by classical or very simple quantum mechanics, and that the apparatus for experimental studies is relatively small scale and inexpensive. Such simplicity makes the field very attractive, especially in view of the extraordinarily broad range of topics which can be investigated with these unusual atoms.

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ARTICLES

 $^{230}\text{Th}/^{234}\text{U}$ dating of travertine from the Bilzingsleben archaeological site**Russell S. Harmon^{*}, Jerzy Głazek[†] & Krystian Nowak[‡]**^{*} Isotope Geology Unit, Scottish Universities Research and Reactor Centre, East Kilbride, Glasgow G75 0QU, UK[†] Institute of Geology; [‡] Institute of Archaeology, Warsaw University, Warsaw, Poland

$^{230}\text{Th}/^{234}\text{U}$ dating of travertine from Bilzingsleben, places an absolute age of $228,000 \pm 17,000$ yr BP on the hominid remains and artefacts at this important archaeological site. Associated floral and faunal fossil assemblages are of interglacial character indicating a depositional climate slightly warmer than at present. These two facts permit correlation of the Bilzingsleben deposits with 'Stage 7' of the marine oxygen isotope record and the penultimate interglacial observed in the European and North American speleothem records.

THE absolute age and stratigraphic position of most of the hominid-bearing deposits of western and central Europe are, at present, uncertain outside the $\sim 40,000$ yr BP limit of conventional ^{14}C dating. Many of these archaeological sites contain coeval inorganically precipitated calcium carbonate deposits which can, under certain circumstances, be dated by natural disequilibrium within the uranium decay series. For example, U-series dating techniques have been used by Turekian and Nelson¹ to date the travertine sequence at Caune de L'Arage, France, at $96,000 \pm 10,000$ yr BP, and by Schwarcz and Debenath² to obtain an absolute age of $146,000 \pm 16,000$ yr BP for skeletal remains in bed 11 of the l'abri Bourgeois-Delaunay rock shelter at La Chaise, France. Here we report $^{230}\text{Th}/^{234}\text{U}$ age determinations for the fossiliferous hominid- and artefact-bearing travertine deposits at the Bilzingsleben site in the German Democratic Republic and briefly discuss the stratigraphic and archaeological significance of this age determination.

The Bilzingsleben site (Fig. 1) is located in the northern portion of the 'Middle German Highlands' about 35 km north of Erfurt in the type-region of the northern European Pleistocene glacial stratigraphic sequence. The archaeological deposits, from which a fossil human skull was first described by von Schlotheim³ in 1818, occur in the Steinrinne travertine quarry

capping an isolated plateau of Triassic (Lower Keuper) marls and dolomites at 35 m above the Wipper River. This plateau has been partially dissected by river entrenchment with four distinct Pleistocene gravel terraces present at elevations of about 30, 20, 10 and 2–5 m within the river valley. Traditionally these terraces have been assigned, in descending order, to the Elsterian, Saalian, Warthian, and Vistulian glaciations⁵. The travertine and associated silts in the quarry fill a lake depression over the highest (30 m) terrace which was formed as the result of subsidence of a karst sinkhole. A schematic section through these deposits is shown in Fig. 2. Directly overlying the gravels of the 30 m terrace are 3–5 m of a slightly eroded silt with furrows in the erosional surface infilled with peatearth and gley soil. Above this is a 4–8 m thick travertine deposit consisting of the following lithologic sequence:

- (1) 30–50 cm of 'sandy' dense, light-brown travertine containing abundant fossils, artefacts and hominid remains;
- (2) 50–80 cm of lacustrine limestone;
- (3) 30–40 cm of 'soft' porous travertine;
- (4) 300–600 cm of 'dense', dark-brown travertine.

Previous studies^{6–8} have shown that calcium carbonate speleothems and travertines can be confidently dated by U-series methods if they contain trace amounts of U, but are free of

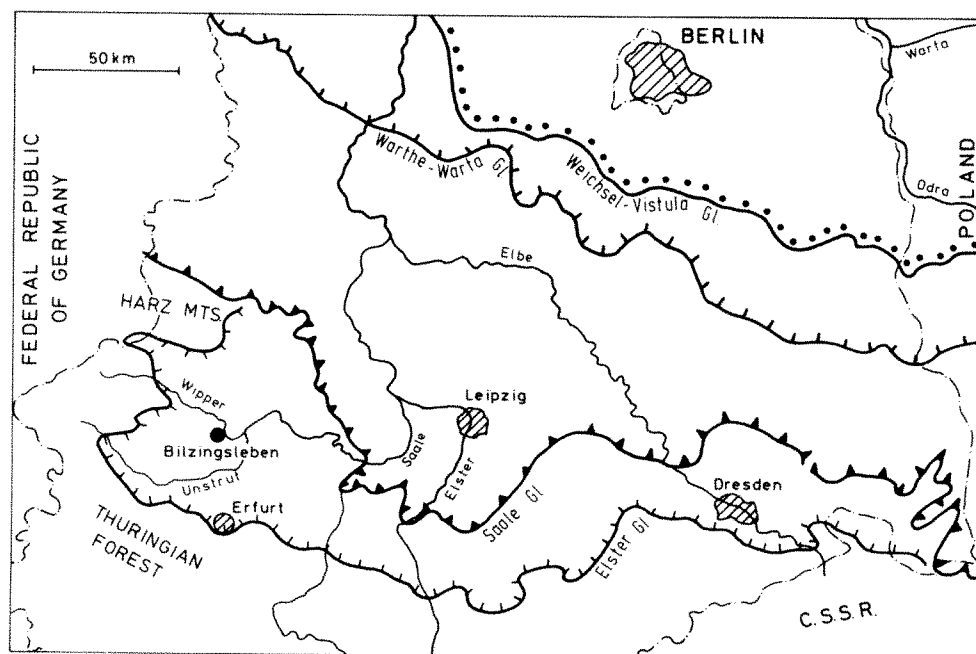


Fig. 1 The limits of continental glaciations in the southern part of the GDR (simplified after Cepek³) in relation to the Bilzingsleben archaeological site.

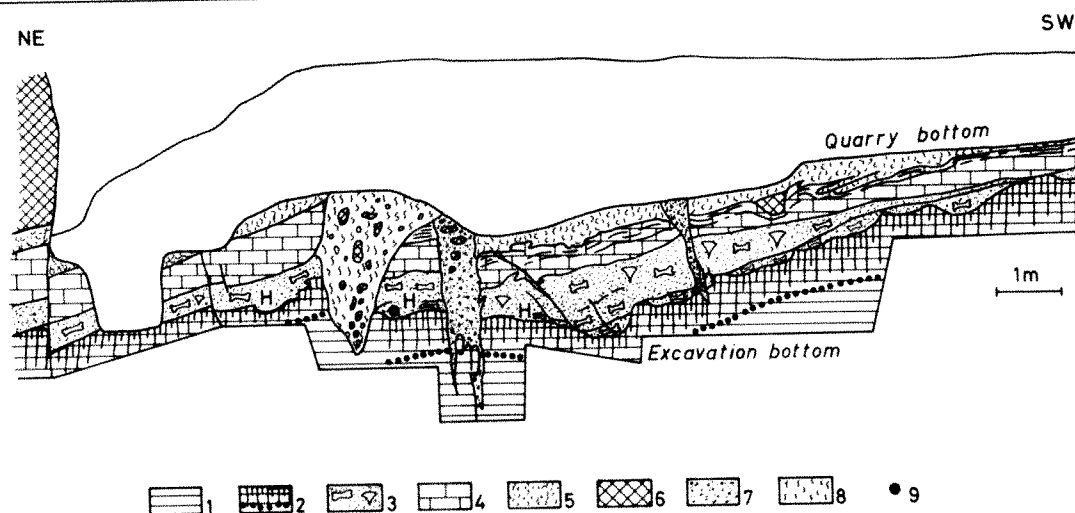


Fig. 2 Schematic cross-section of the travertine sequence in the Steinrinne Quarry near Bilzingseben (simplified after Mania⁵). 1, Silts; 2, soil profile on silts (black on top: gley horizon, vertical dashes: peat earth, black spots in bottom: illuvial carbonate concretions); 3, 'sandy travertine'—hominid—artefact—and bone-bearing layer; 4, lake limestone; 5, soft travertine; 6, hard upper travertine; 7, fissure filling with travertine debris; 8, loess with travertine debris; 9, dated travertine sample; H, location of hominid bone fragments.

detrital Th or Pa. In such instances, absolute dating is possible within the time range ~5,000–350,000 yr BP provided the carbonate is dense and impermeable primary calcite or aragonite showing no signs of diagenetic alteration or post-depositional leaching. The Bilzingsleben travertine which we have dated by the $^{230}\text{Th}/^{234}\text{U}$ method occurs as a dense lens of light brown, micritic calcite within the fossiliferous lower, 'sandy' travertine horizon (Fig. 2). The travertine lens is neither a later (younger) intrusion into the bone-bearing unit, nor is it a detrital clast of unknown age. The stratigraphically younger upper, dark-brown travertine unit was not suitable for dating because it contained a substantial admixture of detrital sediments and iron oxides. Four crushed samples of about 50 g were dissolved in dilute nitric acid and chemically pure U and Th isolated by coprecipitation ion-exchange, and organic solvent techniques^{9,10}. $^{230}\text{Th}/^{234}\text{U}$ ages were calculated from yield and background corrected isotope activities as measured by α spectrometry-pulse height analysis. Analytical data and calculated ages are presented in Table 1. Note the high degree of internal consistency for U concentrations ($4.3\text{--}5.0$ p.p.m.), $^{234}\text{U}/^{238}\text{U}$ ratios ($1.19 \pm 0.02\text{--}1.24 \pm 0.02$), and $^{230}\text{Th}/^{234}\text{U}$ ratios ($0.91 \pm 0.02\text{--}0.93 \pm 0.02$) as well as the high $^{230}\text{Th}/^{232}\text{Th}$ ratios (34–202). This indicates that all ^{230}Th activity in the travertine was authigenic and that it had not been subject to post-depositional loss or addition of U, thus giving us confidence in the $^{230}\text{Th}/^{234}\text{U}$ age determination of $228,000 \pm 17,000$ yr BP.

The stratigraphic age of the Bilzingsleben deposit has been debated for over 50 years. Geological relationships indicate that

the travertine sequence and associated archaeological materials must be younger than Elsterian in age, but there is no other consensus. The interglacial character of the deposit is well established from the abundant floral and faunal fossil material contained in the hominid-bearing, lower travertine unit. Some 10 species of plants, 15 species of snails, and 12 species of vertebrates have been recognised^{5,11–14}. These are listed in Table 2. Ecological interpretation of this fossil assemblage suggests a forest environment with a climate slightly warmer than at present with mean annual temperature elevated by about 2 °C and without winter frost (ref. 15 and A. Sutcliffe, personal communication).

Our radiometric age of $228,000 \pm 17,000$ yr BP permits direct correlation of the Bilzingsleben travertine with 'Stage 7' of the marine foraminiferal oxygen isotope record¹⁴ and the penultimate interglacial period in the North American¹⁷ and European^{18,19} speleothem chronologies (Fig. 3). However, placement of the deposit within the north-central European glacial stratigraphic sequence is more problematic. Although contemporaneity with the Lublin Interglacial in Poland (dated at $245,000 \pm 45,000$ yr BP) (ref. 20) and the Odintsovo (Kaydaki) Interglacial in the western USSR (dated at $227,000 \pm 28,000$ (ref. 21) and $236,000 \pm 25,000$ (ref. 12) yr BP) is suggested on the basis of thermoluminescence ages for these deposits, further correlations of this kind are not possible because of a lack of absolute age data. At some time the Bilzingsleben deposit has been assigned to the Elster-Saale (Holstein)^{5,15,23,24}, Saale-Warthe²⁵, or Warthe-Weichsel (Eem)^{13,26} interglacial periods.

Table 1 U concentrations, isotope activity ratios, and calculated age for the Bilzingsleben hominid-bearing, lower travertine unit

Sample	Description	[U] (p.p.m.)	$\frac{^{230}\text{Th}}{^{234}\text{U}}$	$\frac{^{234}\text{U}}{^{238}\text{U}}$	$\frac{^{230}\text{Th}}{^{232}\text{Th}}$	Age (yr BP)
373	Bulk sample	4.4	0.92 ± 0.02	1.22 ± 0.02	202	$228,000 \pm 17,000$ $-12,000$
374	Fine fraction (270 mesh)	4.3	0.93 ± 0.02	1.24 ± 0.02	34	$234,000 \pm 18,000$ $-13,000$
375	Intermediate fraction (70–270 mesh)	4.5	0.91 ± 0.02	1.19 ± 0.02	75	$223,000 \pm 16,000$ $-11,000$
376	Coarse fraction (>70 mesh)	5.0	0.91 ± 0.02	1.21 ± 0.02	76	$222,000 \pm 16,000$ $-11,000$
	Average		0.92 ± 0.02	1.22 ± 0.02	97	$228,000 \pm 17,000$ $-12,000$

Table 2 Fossil flora and fauna from the Bilzingsleben hominid-bearing, lower travertine unit (after Mania⁵, Wiegers¹¹, Vent¹², Wohlstedt¹³ and Wüst¹⁴)

Plants	Snails	Vertebrates
<i>Buxus sempervirens</i>	<i>Vitina elongata</i>	<i>Castor fiber</i>
<i>Picea excelsa</i>	<i>Zonites verticillus</i>	<i>Trogontherium</i> sp.
<i>Rubus</i> sp.	<i>Patula solaria</i>	<i>Glis glis</i>
<i>Acer pseudoplatanus</i>	<i>Fruiticola umbrosa</i>	<i>Canidae</i> sp.
<i>Betula pubescens</i>	<i>Dibothrium bidens</i>	<i>Ursus arctos</i>
<i>Corylus avellana</i>	<i>Cepaea nemoralis</i>	<i>Felidae</i> sp.
<i>Quercus</i> sp.	<i>Clausilia pumila</i>	<i>Palaeoloxodon antiquus</i>
<i>Salix cinerea</i>	<i>Clausilia tumida</i>	<i>Mammuthus primigenius</i>
<i>Rhododendron</i> sp.	<i>Clausilia filigrana</i>	<i>Equus caballus</i>
<i>Fraxinus excelsior</i>	<i>Orcula dolium</i>	<i>Dicerorhinus mercki</i>
	<i>Isthmia claustralis</i>	<i>Cervus elaphus</i>
	<i>Vertigo moulinsia</i>	<i>Capreolus capreolus</i>
	<i>Vertigo pusilla</i>	
	<i>Azeca schulziana</i>	
	<i>Belgrandia germanica</i>	

It is generally accepted that the Eemian interglacial deposits of Denmark are correlative with 'Stage 5' of the marine oxygen isotope record so that the Bilzingsleben deposit cannot be of last interglacial age. Similarly, a Holsteinian age is ruled out if the arguments of Kukla²⁵ that the Saale ground moraines are at least of 'Stage 8' (and more likely of 'Stage 10') age are accepted. Cepek²⁵ has argued that the Rügen deposits represent an interglacial period between the Saalian and Warthian glacial events, but both the position and nature of this deposit are controversial. The Rügen deposit may, in fact, be correlative with the Bilzingsleben deposits, but there is insufficient evidence to justify such a claim. Therefore, we propose to establish the Bilzingsleben deposit as the type section for the penultimate interglacial period in north-central Europe because the age and interglacial character of the deposit are now firmly established.

Hominid remains from Bilzingsleben were noted as early as 1818 (ref. 4). Since 1969 excavation by the Landesmuseum für Vorgeschichte, Halle a. Saale has produced over 60,000 flint artefacts as well as rock, bone and antler tools⁵. Most of this material is without typology although some Mousterian and Tayacian artefacts have been recognised leading Toepfer²⁶ to assign a Pontiano-Mousterian character (similar to that of

Saccopastore) to the Bilzingsleben artefacts. More recently a Clactonian character for the artefacts has been suggested⁵. Recently discovered hominid bone material^{5,23,24,28} consists of os occipitale, os frontale and one molar. Taxonomic analysis by Stringer *et al.*²⁹ suggests that these hominid fragments represent an archaic form of Middle Pleistocene *Homo sapiens*, although exhibiting some more primitive morphological features which have led Vlček³⁰ to classify them as a new subspecies of *Homo erectus*. The fossils are similar in form to those from Vértesszöllös, bear some resemblance to those from Petralona, Steinheim, and Swanscombe, but are distinctly less evolved than the hominids at Saccopastore, La Chaise and Ehringsdorf²⁹. The samples of the lower travertine unit dated here are directly associated with the Bilzingsleben fossils and artefacts (Fig. 2) and thus an age of 228,000 $\pm 17,000$ yr BP can be assigned to this archaeological site.

This age determination has important implications as regards Middle Pleistocene hominid evolution. It is widely assumed that *Homo erectus* evolved into *Homo sapiens*, but the fossil evidence is equivocal. If Vlček's³⁰ contention that the Bilzingsleben fossils represent a new subspecies of *Homo erectus* and the somewhat dubious U-series ages of ~200,000–220,000 yr BP for the Ehringsdorf lower travertine unit^{8,27,31} are accepted, then it must be concluded that *Homo erectus* and *Homo sapiens* coexisted in central Europe during the penultimate interglacial period or that our present concepts of variation within these hominid species have to be considerably revised. If not, then morphological differences between the primitive hominid form of Petralona, the archaic forms of Bilzingsleben and Vértesszöllös and the more advanced forms of Ehringsdorf, La Chaise, and Saccopastore could represent progressive transitions within a single lineage of *Homo sapiens*. Between-site morphological differences (for example compare Swanscombe and Steinheim with Bilzingsleben and Vértesszöllös) would then be explained in terms of both phyletic change and sexual dimorphism within individual populations which may be superimposed on temporal, geographic and population variations²⁹.

Nevertheless, from the absolute age data presented here, and similar archaeometric studies from France^{1,2} and Greece⁸, it is evident that the archaeological correlations predominantly based on the antiquated concept of only four Pleistocene glaciations must be revised. It is evident that (1) many Middle Pleistocene archaeological deposits (such as that at Bilzingsleben) assigned on meagre and equivocal field evidence to a particular interglacial period, may be miscorrelated and, in fact, represent

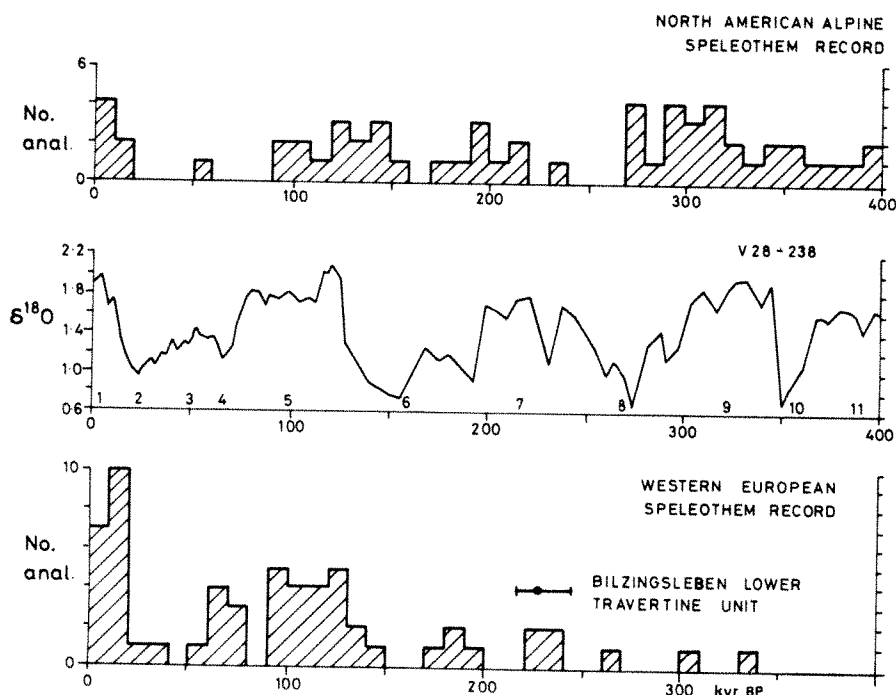


Fig. 3 Comparison of the Bilzingsleben age with the marine foraminiferal oxygen isotope record of Shackleton and Opdike¹⁶, and the North American speleothem chronologies of Harmon *et al.*¹⁷, Atkinson *et al.*¹⁸ and Harmon¹⁹ establishing a penultimate interglacial age for deposition of the Bilzingsleben travertine.

an interglacial event not necessarily recognised in the European terrestrial glacial stratigraphic record, and also that (2) supposed Holsteinian deposits may belong to two or more interglacial periods. Further archaeometric studies of the type described here are required to resolve both of the ambiguities relating to the correlation of European prehistoric cultures and industries (for example, the three different ages for the British Acheulian cited by Collins³²) and are necessary to clarify further the pattern of hominid evolution in Europe (where there is increas-

ing evidence to suggest that *Homo erectus* and *Homo sapiens* may have coexisted during the Middle Pleistocene).

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The crack–seal mechanism of rock deformation

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Many naturally deformed crustal rocks contain mineral-filled extension veins. The crystals making up the vein filling often show a fibrous habit and seem to be built up by a succession of 'crack–seal' increments: the elastically deforming rock fails by fracture, and the walls of the open micro-crack are sealed together by crystalline material derived by pressure solution in the rock matrix.

MANY of the rocks deformed by natural tectonic processes that have occurred in the upper 30 km of the Earth's crust show the development of more or less regular systems of extension cracks and fissures, the fissure spaces filled with silicate or carbonate minerals. The mechanisms by which such veins develop is of special interest to our understanding of crustal deformation. In many orogenic regions extension fissures can apparently give rise to large finite elongations and they can lead to finite strains of several per cent, locally exceeding 50%. The evidence presented here suggests that many, if not most, of these extension veins are formed by an accretionary process involving the formation of a narrow fracture followed by filling of the open space by crystalline material, a mechanism termed 'crack–seal'¹. Geometric features implying this mechanism have been most commonly observed in rocks deformed at metamorphic grades up to upper greenschist facies in environments where stress-induced chemical transfer (pressure solution) of materials seems to be relatively common. The minerals appearing in the vein fillings cover a wide range of mineral species (for example, quartz, calcite, feldspar, actinolite, biotite, and chlorite).

Early stages of vein formation: structure of narrow veins

The first example of the crack–seal process to be described here is taken from pisolitic ironstones of Lower Tertiary age situated

in the frontal folds of the Morcles nappe, one of the important units of the Helvetic nappes, near the village of Champéry, Valais, Switzerland.

The rock is a dense ironstone with sub-spherical, well layered chloritic ironstone pisolites up to 6.0 mm in diameter, and with scattered, isolated, rounded to sub-angular grains of clastic quartz. Figure 1a shows a single crack 45 µm wide passing through matrix, quartz grains and chamosite. The two sides of the crack have been joined together with silicate material; the separate fragments of quartz are sealed together with clear quartz in optical continuity with the broken, inclusion-rich quartz parent, whereas the chamosite grains and ironstone matrix are sealed with fibrous green chlorite. Figure 1b illustrates a vein built up by repetitions of this cracking and sealing process. Eleven small veinlets, each of ~45 µm width, group together to form a compound vein which in the rock specimen appears to the naked eye as a single white vein 0.5 mm in width. The optical control of the successively formed veins by the separated pieces of wall rock is very clear. In the thin sections the successively sealed veinlets give rise to very characteristic 'wing'-like slots of new quartz which in three dimensions have a 'Saturn ring'-like form (Fig. 2). It seems that successive cracks took place along the vein–matrix contacts of a previously sealed crack system, that often a small amount of wall rock material was detached during each successive crack, and that this wall material was preserved as an inclusion trail between the cracks marking successive incremental extensions (Fig. 1c). The cracks

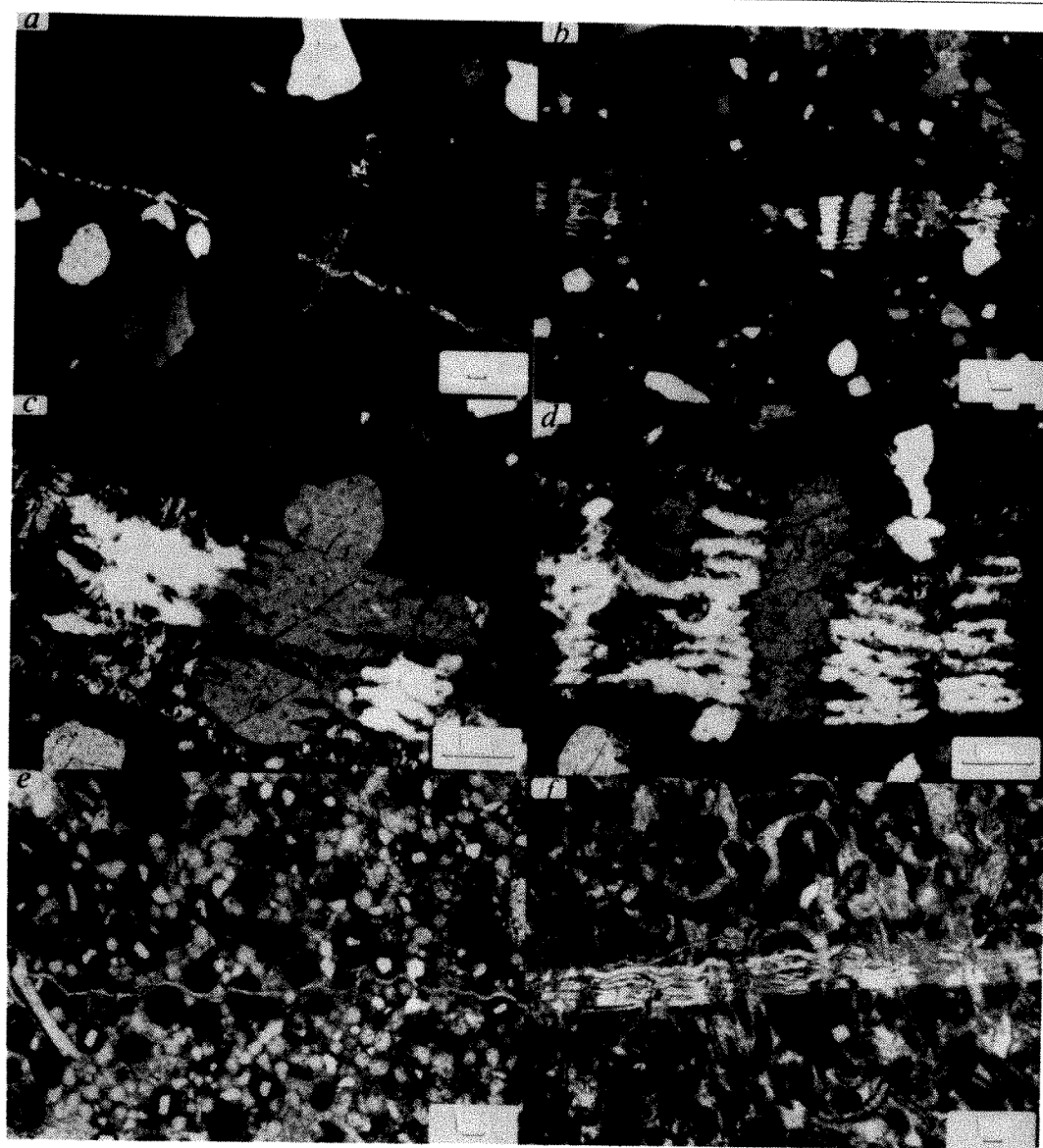


Fig. 1 *a-d*, Thin sections of crack-seal veins in a sandy pisolitic ironstone: *a*, single quartz-chlorite filled crack passing through clastic quartz and chlorite grains; *b*, an array of several small crack-seal veinlets making up a single compound vein; *c*, detail from *b* showing inclusion bands parallel to the main vein wall, the general optical continuity of vein filling with the parent grain in the vein wall, and one crack-seal band (darker grey) slightly misorientated to the other bands; *d*, characteristic 'sawtooth' contacts between elongated fibrous quartz crystals. *e, f*, Thin sections of calcite filled crack-seal veins in oolitic and organic clastic limestones. All scale bars, 100 μm .

occur along the previous vein contacts because this is the overall mechanically weakest surface in the rock.

Although successively grown new quartz strips are generally in optical continuity with the parent quartz, occasionally a misfit of *c*-axis of up to about 3° is seen (Fig. 1*c*). These misfits probably occur because the new growth in a veinlet may be guided by a slightly misorientated seed fragment of broken wall. Where the successive cracks pass along the margin of a quartz grain the inclusion trails so derived are rich in dark iron-rich matrix components, whereas where a crack passes through the centre of a quartz sand grain, the wall-derived inclusions consist mostly of quartz and are visually not so striking. However, the margin of an internally broken quartz grain which has undergone successive fracture and sealing shows a very characteristic saw-tooth form (Fig. 1*d*).

The second example of the crack-seal mechanism is taken from a quite different rock type. The rock is a bioclastic limestone of Jurassic age from the Clariden region of central Switzerland. The limestone sequence is in part oolitic, and in part consists of fragments of shells, crinoids and other organic debris. Figure 1*e* shows a single extension crack passing through an oolitic limestone. The crack sometimes passes directly through the ooids and the matrix, and in places it seems to have been guided by the mechanically weaker boundaries of the oolitic particles. It has been filled with small crystals of clear, crystalline calcite which grow in optical alignment with small

calcite crystals making up the ooids and their matrix. Figure 1*f* shows a succession of calcite sealed microcracks separated by very narrow zones of inclusions of screens of wall rock material, producing an overall vein structure which is remarkably similar to that of the sandy ironstone of the first example. It is especially remarkable that the width of the microveins in the two examples is almost the same, and that the optical orientations of the calcite crystals passing through the composite veinlet aggregate is continuous across the veins, as was the optic orientation of the quartz joining the fragments of broken sand grains.

Crack-seal structure in large veins

The third example of the crack-seal process is a very spectacular one, and illustrates especially well the way that the seal material is added on the walls of the previously formed crack. It comes from highly deformed ferruginous limestones of middle Jurassic age from the Windgällen situated in the autochthonous cover rocks of the Aar Massif of central Switzerland. The limestones contain chamosite oolites and are sufficiently rich in haematite and magnetite to have been mined for their iron content in the latter part of the nineteenth century even though they are situated at an elevation of 2,400 m. The tectonically formed veins are found along planes sub-perpendicular to the direction of maximum finite stretching as determined from the shapes of the deformed oolites^{2,3}. The veins (Fig. 3) consist of fibrous intergrowths of quartz and calcite, the fibres being orientated

perpendicular to the walls of the veins. The veins have the characteristics of antitaxial vein growth⁴—that is, the crystals grow from an approximately centrally located inclusion-rich zone (the median surface) towards the two walls. Some of the individual crystal fibres become larger towards the walls, presumably because they were more favourably orientated for growth than their neighbours.

The most remarkable feature of these antitaxially formed veins is the presence of very regularly orientated lines of solid inclusions, mostly consisting of tiny crystals of chlorite and calcite arranged sub-parallel to the walls of the vein at distances of about 12 μm apart. These lines of inclusions are termed inclusion bands (Figs 3, 4b, 4c). The undulations seen in one inclusion band are more or less parallel to those in the adjacent bands, but the undulations gradually change shape from inclusion bands in the vein centre to those near the vein wall (Fig. 4b). The inclusion bands close to the wall are parallel to the vein-wall contact (Figs 3, 4b). As well as a correspondence of shape between adjacent inclusion bands there is also a striking correspondence in inclusion composition and grain shapes of the individual crystals making up the bands. This is particularly noticeable within calcite inclusions; these form lines of separate, optically parallel and shape related grains aligned sub-perpendicular to the vein wall, termed inclusion trails (Figs 3, 4c, 4d, 4f). In many instances these small grains can be shown to be completely enclosed in the larger fibrous grains of the main host vein crystal, and under the microscope it can be proved that there could have been no direct physical connection with some parent grain located outside the plane of the thin section (such as occurs when a section passes through the edges of the Saturn-ring structure of the crack-seal grains of our first example, shown in Fig. 2). In a few inclusion trails the individual inclusions come very close to each other, and some do form series of more or less continuously linked crystals (Fig. 4d). The composition of crystal components of adjacent inclusion bands change sympathetically (Figs 3, 4c). An inclusion band made up of chlorite particles will change along its length to one made up of calcite crystals, and adjacent bands show identical changes in composition (Figs 4c, 5). The compositional changes along adjacent bands always occur along a line parallel to the inclusion trails. The mineral component changes can be related to the composition of the vein-matrix wall; the inclusion trails of calcite always

root on to the normal granular carbonate of the rock wall, whereas the chlorite inclusion trails always root on to a wall made up of the broken surface of a green chlorite ooid. The trails of optically parallel calcite inclusions lead to an optically identically orientated clear calcite crystal growing on the vein-matrix wall and which itself is optically aligned to a more opaque carbonate fragment of the matrix (Figs 4f, 5).

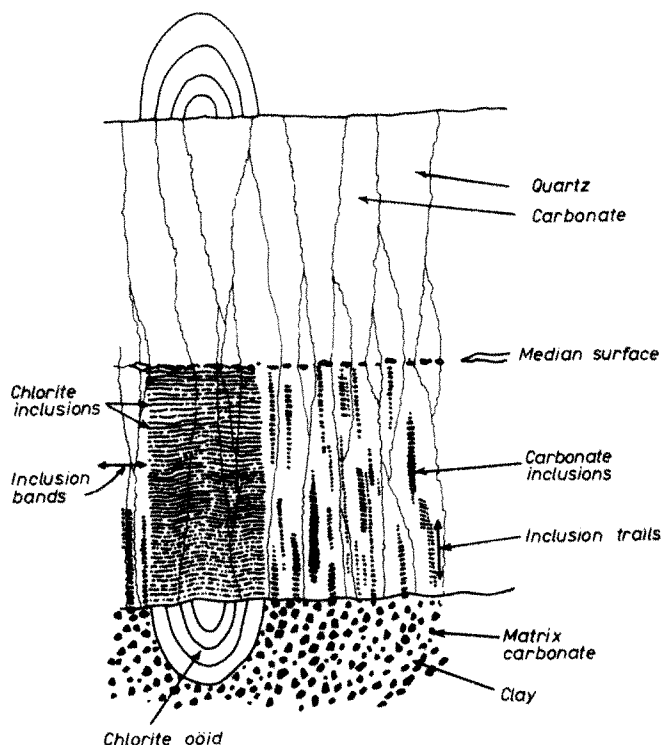


Fig. 3 Schematic diagram to show the main features of quartz-calcite tectonic veins in the oolitic limestone of the Windgällen. The vein on both sides of the median surface shows inclusion bands and inclusion trails, but these have been omitted on the upper side of the median surface so as to show the general shapes of the main vein-forming minerals.

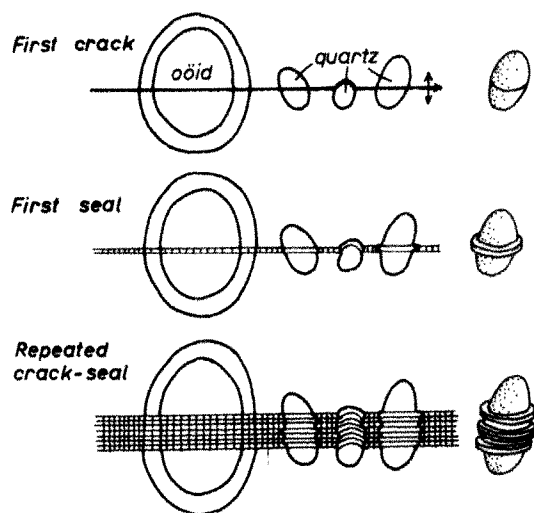


Fig. 2 Schematic development of crack-seal veins. The left-hand side shows the appearance of successive crack-seal veins in cross-section, while the right-hand side illustrates what is believed to be the three dimensional Saturn-ring structure deduced from successive two dimensional thin sections.

The relationships of the large fibrous quartz and calcite crystals forming the main vein filling to each other and to the small crystal inclusions is in main geometric features simple, but, in detail, quite complex. In general the grain-to-grain contacts of the larger crystals are sub-parallel to the inclusion trails (Figs 3, 4d). Calcite and chlorite inclusion trails and bands can be formed in both quartz and calcite forming the larger vein-crystals (Fig. 4f, calcite in quartz, and calcite in calcite in the dark centrally located grain; Fig. 4c, chlorite in quartz and chlorite in calcite). The chemical control on the composition of the inclusion crystals seems to be exerted by the composition of the matrix at the vein-matrix wall and not by the composition of the main vein crystals. Although the contacts of large vein-forming crystals are generally almost parallel to the inclusion trails in several examples the inclusions forming a trail transect these contacts passing from one large main vein crystal to another (Figs 3, 4d, 5). The quartz-quartz, quartz-calcite and calcite-calcite contacts of the main vein crystals often show a saw-tooth shape (Figs 4b, 4d, 5), the dimensions of the irregularities being the same as those of the inclusion band spacing. Although the large vein-forming crystals linking across the inclusion bands are generally optically continuous (Figs 4e, 4f), some slight irregularities do occur in the optical parallelism between certain inclusion bands. Variation in *c*-axis orientation

of up to 3° gives rise to a gently undulating dark-line polarisation colour appearance under crossed polars (Fig. 4c), and in a few instances slight variations of *c*-axis orientation in a single large individual crystal seems to be the result of plastic deformation after crystallisation (Fig. 4b).

The remarkable geometric features of these veins have been described in detail because they seem to provide a key to the understanding of vein-growth by the crack-seal mechanism. The inclusion band and inclusion trail structure seems to be best explained with recourse to progressive incremental cracking along the vein-matrix contacts, followed by a sealing together of the crack walls by crystalline material precipitated from a fluid phase. The successive microcracks were filled with fluids containing components in solution which were chemically closely related to the components forming the main body of the rock, and which were probably derived by local chemical solution and transfer (pressure solution). Some of these components (Ca^{2+} , CO_3^{2-} , Mg^{2+} , Fe^{2+} , Fe^{3+} , Al^{3+} , Si^{4+}) were then precipitated by syntaxial overgrowth on the crystals making up the microcrack walls as new formed calcite and chlorite. Some of the wall rock calcite crystals were favourably orientated for rapid growth (see, for example, Fig. 5, calcite in trail C_{14}) and could form a complete wall to wall link between the two sides, whereas others (Fig. 5, crystals in trails C_{11} , C_{12} , C_{13} , C_{15}) did not grow so fast and were therefore cut off by other faster growing crystal components before they could form a cross-link. The main crystal components which make up the continuous long fibres

(Fig. 5, quartz Q_1 , Q_2 , Q_3 and calcite C_1) were nucleated in the first formed crack, and because newly forming cracks always develop along the mechanically weak contacts of earlier formed veins, successive additions of new material lead to fibrous growth of these crystals away from the initial vein (antitaxial growth). These crystals can only grow sideways parallel to the vein at the expense of less favourably growth orientated neighbours. These space competing neighbours might be of the same mineral species (Fig. 5, quartz grain Q_2 growing at the expense of grains Q_1 and Q_3) or of a different species (calcite grain C_1 growing at the expense of quartz grain Q_3 and including unfavourably orientated calcite C_{15} on an inclusion trail). The crystallisation of the main vein component usually leads to a complete solid seal across the walls, but occasionally one finds remnants of fluid phases preserved as small fluid-gas bubble inclusions or negative crystal fillings. The fact that crystallisation of solid proceeds to form a complete wall-to-wall seal implies either that fluid containing the source of solid in solution must continually flow through the fissure, or that there is a continual replenishment of solid phase from the vein walls into the fluid phase occupying the progressively filling fissure. Once the sealing of the walls of the microcrack is complete, tectonic stresses are able to be transmitted across the vein and its walls once more. The intensity of these stresses builds up until a critical level of stability is achieved. The vein matrix contact then fails and a new fracture space is developed whose dimensions are controlled by the elastic recovery of the stressed walls. The

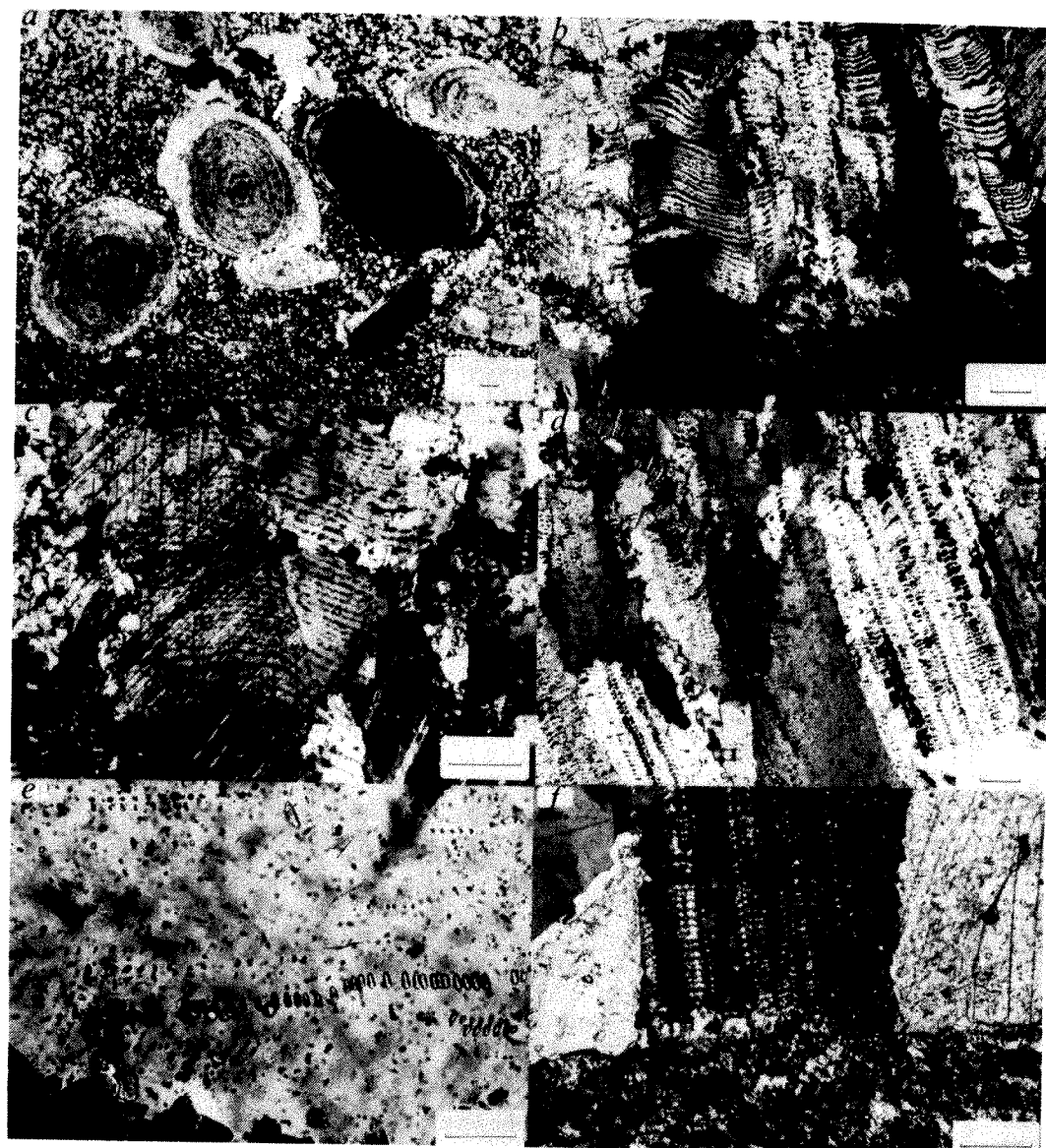


Fig. 4 Thin sections of crack-seal veins from the Windgällen, all taken with crossed polars. *a*, The chamosite ooids with characteristic concentric structures in a dark iron-rich matrix containing pale carbonate grains; *b*, inclusion bands parallel to the vein wall—chlorite inclusion bands occur where the wall of the vein is occupied by an ooid, whereas calcite inclusion bands are found where normal matrix carbonates are found along the vein walls (see Fig. 5); *c*, inclusion bands passing through large calcite and quartz crystals and changing in composition from chlorite components to calcite components (left to right); *d*, inclusion trails of calcite passing through quartz fibres; *e*, detail of calcite inclusion trails in quartz showing the remarkable shape similarity of the individuals making up an inclusion trail; *f*, calcite inclusion trails linking on to wall rock calcites with the same optical orientation. All scale bars, 100 μm .

Fig. 5 Schematic diagram showing the main features of the inclusion band and inclusion trail structures in the Windgällen veins. Calcite crystals with line ornament, quartz crystals stippled.

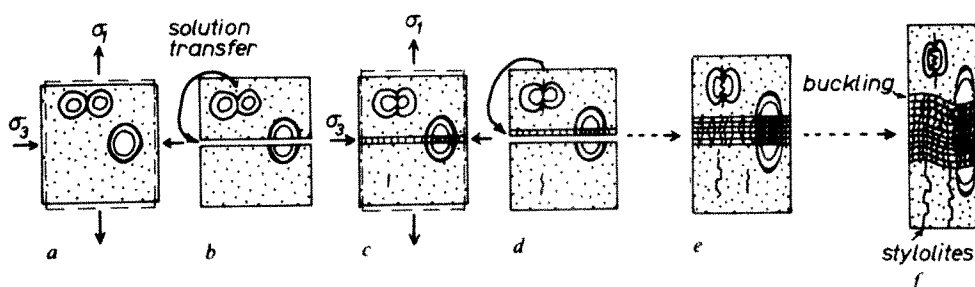
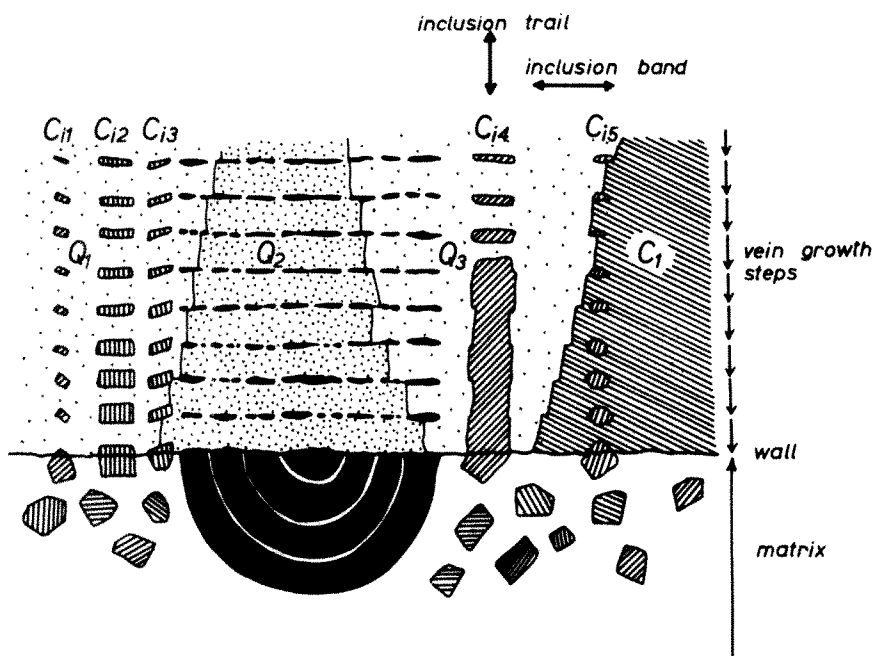


Fig. 6 Diagram showing the sequence of events leading to elongation of a rock by the crack-seal mechanism: *a*, elastic strain accumulation (σ_1 and σ_3 are greatest and least principal tensile stresses); *b*, development of first fracture and release of elastic strains in matrix, solution transfer

of material from walls into microcrack; *c*, seal of vein walls and further elastic strain accumulation; *d*, development of second crack and release of elastic strains in vein walls, further solution transfer with development of stylolitic surfaces; *e*, repeated crack-seal microcracks forming a compound vein; *f*, large finite shortening leading to vein buckling and the formation of stylolites both outside and inside the compound veins.

process of sealing the walls is repeated, and the crack-seal cycle is repeated indefinitely providing that sufficient tectonic stress accumulates for cracks to develop or that sufficient material is available in the solution which fills the cracks to seal the walls together. The Windgällen veins described here show more than 500 increments in a total vein width of 7.5 mm.

Conclusions

Figure 6 gives a review of the sequence of events by which a large overall extension by the crack-seal mechanism can be achieved. Tectonically induced elastic strains reach a critical level, and then fracture occurs (Fig. 6, *a* and *b*). The mechanism which brings about tensile fracture is probably that termed hydraulic fracturing⁵⁻⁷. There is strong evidence from the later precipitation of materials in the fracture that a fluid phase was present during fracturing, and it is well established that tensile fracturing can be initiated when the fluid pressure equals the tensile strength of the rock. Fluid-filled open microfractures are formed which are later filled with crystalline material derived by pressure solution in the rock matrix and chemical transfer of this material into the low pressure fluid-filled space (Fig. 6*c*). The

process is repeated and new material is added antitaxially so that the vein walls of the initial vein accumulate new material. The outward growth occurs because, statistically, there are equal chances of new cracks forming and subsequent new sealing material developing on both vein-matrix walls (Fig. 6*e*). At high finite strains (say greater than 10% shortening) the veins themselves have to shorten. Vein shortening is accomplished by buckling or by the development of tectonic stylolites along the grain boundaries of adjacent previously crystallised fibres (Fig. 6*f*).

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The use of synchrotron radiation in time-resolved X-ray diffraction studies of myosin layer-line reflections during muscle contraction

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Experiments on striated muscle have been carried out at the EMBL Outstation at DESY, Hamburg, using the electron-positron storage ring DORIS as a high-intensity X-ray source. The low-angle reflections from the myosin cross-bridges could be recorded more than 1,000 times more rapidly than with the best conventional X-ray sources, and could be followed during contraction with a time resolution of 10 ms.

DETAILS of the molecular mechanism which produces the sliding force between actin and myosin filaments during muscle contraction have proved difficult to establish. It has long been suggested^{1,2} that the force is developed by moving projections or cross-bridges on the myosin filaments which may undergo a cyclical process of attachment and detachment with sites on the actin filaments, but direct structural evidence for this mechanism is inherently difficult to obtain. Surviving muscles give informative low-angle X-ray diffraction diagrams³⁻⁵ which, in principle, can reveal details of cross-bridge behaviour during contraction. However, the use of this approach has previously been restricted by the long exposure times needed to record the patterns on film, although much useful information has been obtained⁴⁻⁹. Recently the use of increasingly efficient and sophisticated X-ray counters and electronic data handling systems has made it possible to carry out time-resolved measurements on the stronger equatorial and meridional reflections from striated muscles with a time resolution in the 10 ms range^{10-16,28,29}. This is the time scale required to make useful comparisons with normal tension development and decay in the muscles commonly used (for example, frog sartorius), but is too slow to study faster transient processes. Moreover, the off-meridional pattern of myosin layer-lines, which arises from the helical arrangement of the cross-bridges, is too faint to be recorded with adequate time resolution, even using very long contraction series, if normal laboratory-based X-ray sources are used.

For this and analogous reasons, there has recently been considerable interest in the use of synchrotron radiation as a high-intensity X-ray source for diffraction experiments^{17,18}. We report here some observations made at the EMBL Outstation at DESY, Hamburg, using the electron-positron storage ring DORIS.

Use of a two-dimensional TV detector

In our initial experiments we were concerned to establish whether or not the loss of layer-line intensity, which had earlier been observed (using film and laboratory X-ray sources) to occur during a very long series of tetanic contractions, was a genuine effect occurring during an unfatigued contraction in a freshly dissected muscle. We used a frog sartorius muscle, maintained in an oxygenated Ringer solution in the usual manner^{4,8} and stimulated directly by platinum electrodes lying along its length on either side. Tension was measured and recorded electronically. A focused and monochromatized X-ray beam was produced from the incident radiation from the storage

ring by a camera designed by Rosenbaum and Harmsen¹⁹, comprising an eight-element, totally reflecting glass mirror of total length 160 cm and a germanium curved crystal monochromator. With this apparatus, a good-quality layer-line pattern from muscle could be recorded on film in a few minutes, compared with about 24 hours for a similar quality pattern using a high-power rotating anode X-ray tube. In the actual experiments, however, the pattern was recorded on an X-ray image intensifier-TV detector^{20,21}, as this enabled good layer line data to be collected with a total exposure time of only 1 s. The two-dimensional nature of the detector had the great advantage that the whole of the X-ray pattern was available for inspection and measurement, and indeed the myosin layer-lines could be viewed directly in 'real time' on a monitor integrating over about 1 s. In this way, it was possible to see the pattern disappear as the muscle was stimulated tetanically for 1 or 2 s, and then reappear as soon as stimulation ceased. However, when collecting time-resolved data, only the pattern corresponding to one particular

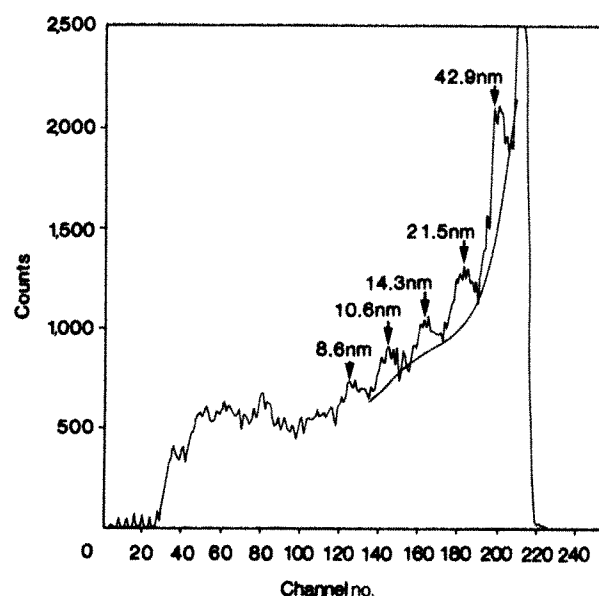


Fig. 1 Layer-line pattern from resting frog sartorius muscle at 10 °C recorded on the position-sensitive counter. Background fitted using a fourth order polynomial; total exposure time 3 s. Layer-line spacings are indicated.

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'time-slot' could be recorded in a given series of experiments because of readout time limitations with this type of detector.

To investigate the behaviour of the pattern during single twitches, we recorded the pattern during a time interval of 150 ms and in each run of 6–10 twitches this 'slot' was set at the same time during contraction. A total exposure time of 1–1.5 s was sufficient to record a good-quality pattern. The run was then repeated for a different time slice. Within this limited time resolution it could be seen that the disappearance of the layer-line pattern at the beginning of the twitch and its return during relaxation followed a time course very similar to tension development and its decay. This could be observed in quite short contraction series in which there was no doubt that the muscle was in good condition throughout and where there was none of the weakening of the resting layer-line reflections that we have observed in longer series of tetanic contractions, especially at lower temperatures; nor did we see the delayed return of off-meridional layer-line pattern after contraction apparent in very long contraction series¹³. Thus, the experiments showed unambiguously that the loss of the layer-line pattern is a specific and reversible accompaniment of normal contraction.

It was also noticeable, as observed previously⁴, that the decrease in intensity of the 14.3-nm meridional reflection was often much less marked than that of the off-meridional layer lines. Nevertheless, such muscles showed almost a complete disappearance of the 'forbidden' 21.5-nm meridional reflection during the peak of the twitch, and its reappearance during relaxation.

Intensity measurements with a one-dimensional detector

To compare the time course of the layer-line changes more accurately with that of tension, a different system was used. This used a position-sensitive one-dimensional proportional counter of the delay-line type, designed by Gabriel²². This was positioned over the muscle pattern with its axis parallel to the meridian but displaced sideways from it so as to record the strongest parts of the set of myosin layer-lines. When filled with a xenon-CO₂ mixture at 2 atm, the counter could record the first few layer-lines satisfactorily in a few seconds, giving a total of several thousand counts in the first layer-line after background stripping. (For stronger parts of the pattern, the beam had to be attenuated.) The delay line output was decoded using a direct time-digitising device (modified CERN modules DTD164 and 211)²³. The latter are read out by an auxiliary CAMAC crate controller designed by C. Boulin. This module stores the one-dimensional patterns in a memory linked to a PDP11/45 computer²⁴ in 10-ms time bins via a bin switching device designed and built by E. Dorrington. The time slots are synchronised electronically with the stimulation of the muscle. Details of this system will be described elsewhere. The X-13 camera²⁵ used in these later experiments is another mirror monochromator device again using an 8 × 20-cm section mirror but with a triangular-shaped monochromator which is bent into a section of circle by applying pressure at the apex²⁶. The specimen-to-detector distance was approximately 2.2 m.

With the storage ring operating in the dedicated mode at 3.185 GeV and 70–90 m/a (positrons only), with 480 bunches, total counting rates over one quadrant of the layer-line pattern were in excess of 10⁵ c.p.s. and adequate data could be collected in 10-ms time bins from a series of a few hundred single twitches spaced at 30-s intervals. Thus, a typical experimental run would last 2–3 h and gave a few seconds of total exposure time in each time bin.

In these experiments (and in the earlier ones) a fast-acting lead shutter was arranged so that exposure of the muscle to the X-ray beam was minimised, being kept to 2 or 3 min even in the longest runs. No evidence of radiation damage was apparent in these experiments. However, in test exposures exceeding 10–15 min, deterioration of both the X-ray diagram and the microscopic appearance of the region of the muscle in the X-ray beam was

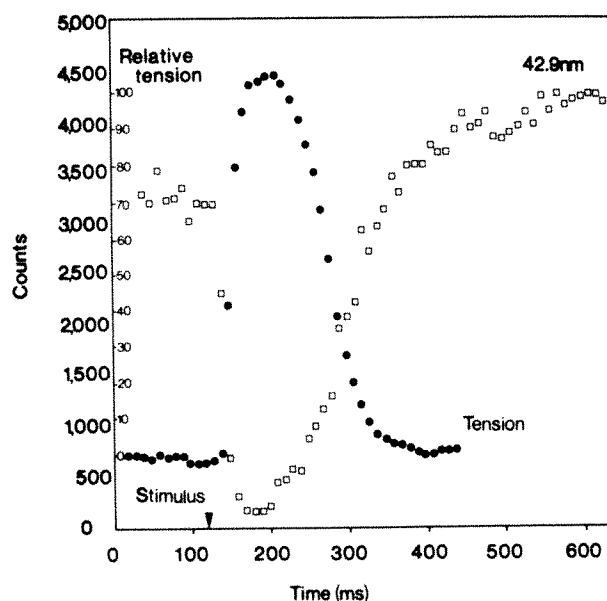


Fig. 2 Changes in the 42.9-nm layer line intensity (□) and the relative tension (●) during a twitch in frog sartorius muscle at 10 °C. Data are summed from 900 twitches, each time slot being of 10 ms duration, that is, corresponding to 9 s total exposure. Time at which electrical stimulus applied to muscle is shown by an arrow. Incident intensity at specimen was reduced by a factor of 3 (using aluminium foils) because of total counting rate limitations (later overcome) in the electronic circuits at the time of this experiment.

quite obvious. As such radiation effects have not apparently been observed with glycerinated insect flight muscle, it may be that the radiation-sensitive component resides in the calcium-releasing mechanism.

Data could also be collected from a much smaller number of twitches if longer time slots were utilised (for example, 50 ms), but no changes in the behaviour of the pattern were noted over the experimental times used. Meridional data were also collected, usually from considerable shorter series of twitches, as the patterns are much stronger and have a higher signal-to-noise ratio.

The layer-line reflections are superimposed on a steeply sloping background (see Fig. 1). A computer program was used to draw in the best fit (least squares) of a polynomial to the background levels seen between the successive layer-line peaks. It was found that a third (or sometimes fourth) order polynomial gave a satisfactory fit. If a separate fitting operation was carried out for each set of data in successive time slots, it was noticeable that a slight increase in the background level occurred during contraction. However, the most striking change was the large decrease in all the layer-line intensities during the time that the muscle was developing tension. The time courses of the changes in all the layer-lines do not seem to differ significantly and for simplicity we will consider only the first and strongest of these, that at 42.9 nm. The results of a typical run at 10 °C are shown in Fig. 2, where the intensity of the reflection is plotted as a function of time after the stimulus of the muscle (by a single maximal shock), in 10-ms time channels. The isometric tension developed by the muscle is shown on the same time scale. It can be seen that the reflection begins to decrease in intensity within 20 ms of stimulation, falls almost to zero intensity (in some cases) by about 70 ms, and then returns to its resting value (or even slightly more on several occasions) after about 200–250 ms.

The relationship between change in intensity and tension is shown more clearly in Fig. 3, where the change in each of them is plotted as a percentage of the maximum change occurring. It can be seen that both during the onset of activity and during relaxation, isometric tension and change in X-ray intensity follow a markedly similar time course; this was the case in all the records we obtained (six good runs so far) although in one or two

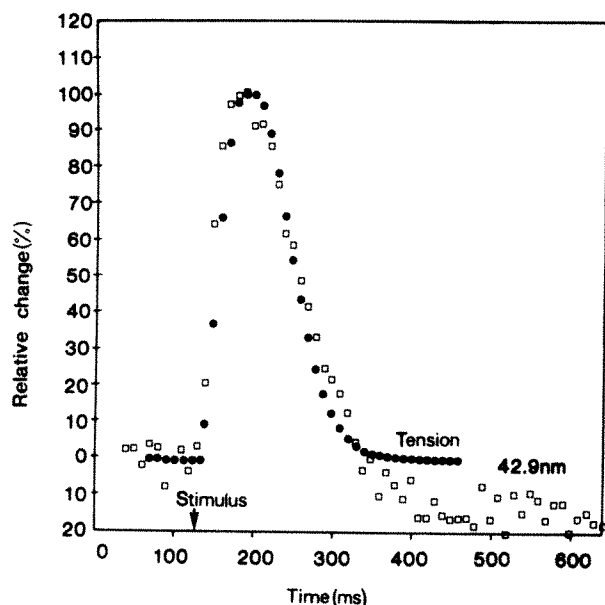


Fig. 3 Changes in the 42.9-nm layer line intensity (\square) and tension (\bullet) plotted as a percentage of maximal change. Other conditions are the same as in Fig. 2 except that the total exposure for each 10-ms time slot was only 3 s.

a very slight lag in the X-ray pattern (≤ 20 ms) was noted during relaxation. We do not have sufficient data, however, to establish whether or not the intensity change leads the tension change during the rising phase of tension by a few milliseconds at this temperature, as it does in the case of the equatorial reflections¹⁴.

Measurements of the intensity changes in the 14.3-nm meridional reflection in exactly the same conditions showed a rather interesting effect (Fig. 4). The change in intensity of the reflection, plotted again as a function of time after stimulus, exhibited a pronounced biphasic behaviour. The initial decrease in intensity took place with essentially the same time course as that of the layer-lines, but this was followed by a slight recovery of intensity during the peak of the twitch and then a large decrease in intensity again during the relaxation phase. Thus, the 14.3-nm intensity fell to its minimum value when the tension and layer-line intensities had almost returned to their resting values, and it returned to its resting value with a pronounced lag behind tension recovery. This lag has been seen in many previous experiments (using laboratory X-ray sources) and the biphasic fall in intensity has also been seen previously, although less frequently. This effect might be due to a longitudinal disordering of the myosin filaments, which in a resting muscle are arranged with their sets of cross-bridges in register. This would have a much larger effect on the intensity of meridional reflections than on the off-meridional layer-lines, and the disordering might be maximal during the relaxation phase, when different regions of a muscle relax at somewhat different rates and when disorder of the sarcomere pattern is much more frequent and pronounced than at other times during the twitch. We hope to investigate the phenomena further in future experiments.

Muscles at 'no-overlap' length

We have also carried out some preliminary experiments on the behaviour of the layer-line pattern in frog semitendinosus muscles stimulated at very stretched lengths where overlap of thin and thick filaments is either completely abolished or very greatly reduced. In earlier work using film and laboratory X-ray sources, in which very long tetani (7 s) had to be repeated many hundreds of times over a period of a day or more to record the patterns, a substantial decrease in layer-line intensity was observed, but it was not known whether this was due to an activation mechanism in the myosin filaments themselves, or whether it was associated with a progressive disordering of the

sarcomere structure allowing actin-myosin interaction still to occur at these extended sarcomere lengths^{7,9}. In the present experiments, using both the image-intensifier system and the position-sensitive counter system, we were able to record the layer-line pattern given by these stretched muscles by summing over as few as 10 1-s tetani, so that the possibility of the 'creep' artefact was very much reduced.

In all our experiments so far we have found a very large reduction in the extent of decrease of the layer-line intensities from very stretched muscles during stimulation compared with that seen in similar muscles at normal sarcomere lengths, and in most of the experiments no decrease at all in intensity was observed. For technical reasons it was not possible to determine sarcomere lengths during these experiments, in which active tension was reduced to about 10% of its rest-length value, but as the ability to develop full tension (and to show the normal layer-line changes) was restored when the muscles were returned to resting length, it seems likely that the muscles were close to the non-overlap length and in good condition. Thus, we conclude provisionally that interaction with actin is necessary for helical disordering of the myosin cross-bridges to occur following stimulation. This agrees with recent observations showing an absence of radial cross-bridge movement at no-overlap sarcomere lengths, as judged by the absence of change in the equatorial X-ray diagram¹⁶.

Interpretation of intensity changes

The principal finding we will discuss here is the large decrease in intensity of the layer-lines at resting sarcomere lengths. When this was first seen in the earlier experiments using film and long series of tetanic contraction^{4,5}, it was interpreted as indicating a disordering of the regular helical arrangement of myosin cross-bridges (in resting muscle), brought about by their interaction with actin during contraction. This interpretation remains a very plausible one, and is reinforced by the fact that the change is so closely correlated temporally with tension development, and that the bridges return to their normal helical order around each myosin filament immediately tension disappears (at least in relatively short series of twitches at 10 °C). During the onset of tension, although the ends of the muscles were held fixed, there would be significant internal shortening of the muscle against series elastic elements, equivalent to at least a 2 or 3% change in

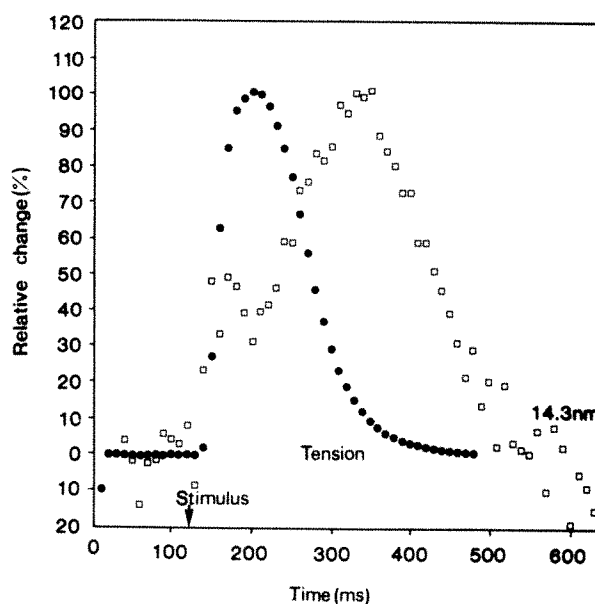


Fig. 4 Changes (plotted as a percentage of maximal changes) in the 14.3-nm meridional reflection (\square) and tension (\bullet) during a twitch from frog sartorius muscle at 10 °C. Total exposure is 1.5 s in each 10-ms time slot.

muscle length. This would correspond to several hundred angstroms of sliding movement between the overlapping actin and myosin filaments. Thus, if this movement was produced by a change in tilt of attached cross-bridges, we would expect to find the population of attached bridges distributed over all the possible angles of tilt, given the asynchronous nature of the interaction. This could explain why neither myosin type nor labelled actin type layer-lines are visible.

We consider, then, that these rapid and reversible changes in the myosin layer-line pattern during contraction provide further

good evidence that contractile force is produced by cross-bridges which attach to actin in the active muscle and undergo some form of tilting and pulling movement, as suggested previously²⁷. The experiments also demonstrate the usefulness of synchrotron radiation as a high-intensity X-ray source for time-resolved studies: it should also be possible to extend the scope of such studies considerably.

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Order and intracellular location of the events involved in the maturation of a spliced tRNA

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Microinjected frog oocytes were used to analyse the RNA processing steps which lead to the appearance of a mature cytoplasmic tRNA^{tyr} molecule. The results show that removal of the intervening sequence from within a yeast tRNA^{tyr} precursor, excision of extra 3' and 5' nucleotides, addition of a 3'-terminal CCA and modification of at least seven ribonucleotides all occur in the nucleus before the tRNA^{tyr} is transported to the cytoplasm. Moreover, we find that the ribonucleotide modifications occur in a strict order which precisely correlates with the size alterations of the tRNA^{tyr} precursor.

THE transcript of most eukaryotic genes is an RNA precursor which must be processed post-transcriptionally to yield a functional gene product¹⁻³. In the case of transfer RNAs the RNA processing or maturation includes size alterations and base modifications of an RNA precursor as well as transport of the RNA from the nucleus to the cytoplasm (reviewed by Smith in ref. 4). Furthermore, in the light of the discovery of intervening sequences in some tRNA precursors⁵⁻⁹, it is now clear that tRNA maturation sometimes involves the removal or splicing of intervening RNA sequences. At present, the intracellular location of the precursors and mature tRNAs and the relative order of the various processing steps is virtually unknown for eukaryotic tRNAs. This article describes how and where in the cell the RNA transcript of a yeast tyrosine tRNA gene is processed into a mature cytoplasmic tRNA.

The DNA sequences of four of the eight tyrosine tRNA genes (tDNA^{tyr}) of the yeast *Saccharomyces cerevisiae* have been determined (ref. 5 and P. Phillipsen, J. R. Cameron and R. W. Davis, personal communication). These data have shown that the tRNA^{tyr} genes contain a 14 base-pair intervening sequence, adjacent to the anticodon, which is not present in the mature tRNA. Previous studies on the expression of this gene in microinjected frog oocytes showed that the tDNA^{tyr} is first transcribed as a precursor RNA which has an extension at the 5' end of the mature molecule and an intervening sequence, but lacks a 3'-terminal CCA¹². In this article we analyse the processing of

the tRNA^{tyr} precursor in detail, focusing on the intracellular location of the RNA processing events. We conclude that (1) tRNA precursors with immature ends (having 5' leader and 3' trailer sequences) are located only in the nucleus, (2) removal or splicing of the intervening sequence occurs in the nucleus or at the nuclear membrane and (3) size alterations and base modifications occur in a strict temporal order.

Expression of cloned tRNA genes in injected oocytes

Amphibian oocytes have been used extensively as a biological test system for the expression of purified mRNAs and more recently for cloned genes (reviewed in ref. 13). It has been shown that cloned DNAs are accurately transcribed following injection into the nucleus of intact *Xenopus* oocytes¹⁴⁻¹⁸ or after addition *in vitro* to oocyte nuclear extracts¹⁹⁻²². Correct processing of the RNA transcripts in this system is demonstrated by the fact that oocytes injected with a cloned tRNA gene synthesise a tRNA precursor and process it into a mature tRNA having the correct size, sequence and even some of the appropriate base modifications^{12,18}. Moreover, the transcripts from the injected genes are translated into proteins in some cases, for example, SV40 DNA and sea urchin histone DNA^{23,24,46}.

In using this 'living test tube' to study the transcription of cloned DNAs many copies (usually about 10⁸) of one gene are injected into each oocyte. Consequently, the unusual situation exists in which most of the cells' newly synthesised RNA is

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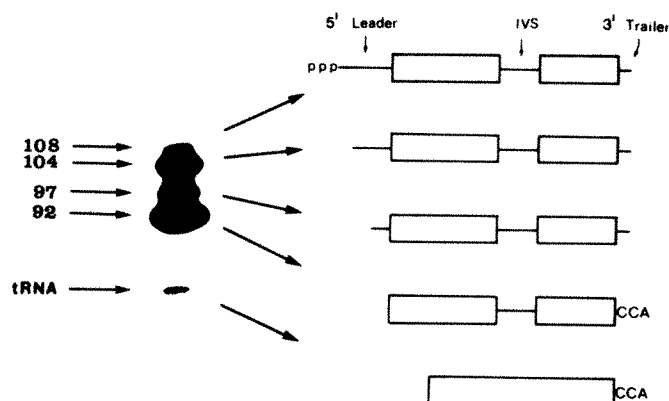


Fig. 1 Transcriptional products of $tDNA^{tyr}$ in injected oocytes. Oocytes were injected with $tDNA^{tyr}$ (plasmid pYTC (ref. 12), 5 ng per oocyte) and ^{32}P - α -GTP (10 mCi mol $^{-1}$), incubated for 6 h and the ^{32}P RNAs were extracted and electrophoresed in a 10% acrylamide gel as described¹⁸. The autoradiogram (left) shows the $tDNA^{tyr}$ precursors and mature $tRNA^{tyr}$ with their approximate lengths (± 4 bases). The structures of the precursors (right) were determined by comparing the RNA fingerprints (as in Fig. 4) with the DNA sequence as described in refs 5 and 12. The 5' leader is about 19 bases long, the intervening sequence is 14 bases long, the 3' trailer is 2 or 3 U, and the mature tRNA is 78 bases long. For the RNA sequences and a possible secondary structure of the precursors see ref. 12.

transcribed from just one type of gene: the injected template. When, for example, yeast $tDNA^{tyr}$ is injected into oocytes the transcripts from this gene, which include mature $tRNA^{tyr}$ and four $tRNA^{tyr}$ precursors, comprise up to 80% of the cells' newly synthesised RNA. This readily allows one to determine the structure of the tRNA precursors.

The intracellular location of the precursors and their processing enzymes can also be determined with this injection system²⁵. The *Xenopus* oocyte is such a large cell (~ 1.2 mm diameter) that its nucleus or germinal vesicle (~ 0.4 mm diameter) can be isolated by manual dissection. Thus, the newly synthesised RNAs present in the nucleus and cytoplasm can be analysed separately. Moreover, so many $tDNA$ transcripts are produced by one injected oocyte ($\sim 2,000$ d.p.m. of ^{32}P -labelled RNA h $^{-1}$) that these transcriptional products can be analysed using a single injected oocyte.

tRNA Precursors with 5' leader and 3' trailer sequences do not leave the nucleus

When oocytes are injected with $tDNA^{tyr}$ and radioactive ribonucleotide triphosphates five main RNA species are synthesised. The structures of the $tRNA^{tyr}$ precursors, determined by RNA fingerprinting techniques, are shown schematically in Fig. 1. Note that the three longest precursors (108, 104, and 97 bases) all contain extensions at the 5' and 3' termini of the tRNA and an intervening sequence in the coding region. By a stepwise removal of the 5' leader sequence these precursors are processed into a 92-base precursor which has a mature 5' end, retains the intervening sequence, and has a 3' CCA. Finally, the 14 base-pair intervening sequence is removed giving rise to mature $tRNA^{tyr}$.

The intracellular location of the $tDNA^{tyr}$ transcripts was determined by dissecting oocytes which had been previously injected with $tDNA^{tyr}$ and [α - ^{32}P]GTP. In these experiments the RNAs present in the nucleus and in the cytoplasm were determined by analysing single oocytes. The results show a partitioning of the transcripts between the nucleus and cytoplasm (Fig. 2). All of the $tRNA^{tyr}$ precursors which contain 5' leader and 3' trailer sequences are found exclusively in the nucleus. The 92-base precursor which contains the intervening sequence, but has mature 5' and 3' termini, is found in both the nucleus and cytoplasm. The mature $tRNA^{tyr}$ (78 bases) is found predominantly, if not exclusively, in the cytoplasm.

After 2.5 h of $tDNA^{tyr}$ transcription most of the transcripts are still in the nucleus and little if any mature $tRNA^{tyr}$ has appeared (Fig. 2). By 6 h some mature $tRNA^{tyr}$ is detected in the cytoplasm and the 92-base precursor is the most prevalent transcript. By 24 h more mature $tRNA^{tyr}$ has accumulated in the cytoplasm, but the 92-base precursor is still the major transcriptional product.

From these results we conclude that tRNA precursors with extra tracts of RNA at their termini are localised in the nucleus. One trivial explanation for this observation is that the larger precursors are rapidly processed and/or degraded upon entering the cytoplasm. This possibility has been rendered improbable by experiments in which the 108-, 104- and 97-base precursors were injected directly into the oocyte cytoplasm and found to be stable. The nuclear segregation found for these $tRNA^{tyr}$ precursors has also been observed following micro-injection of other tRNA genes, including nematode leucine

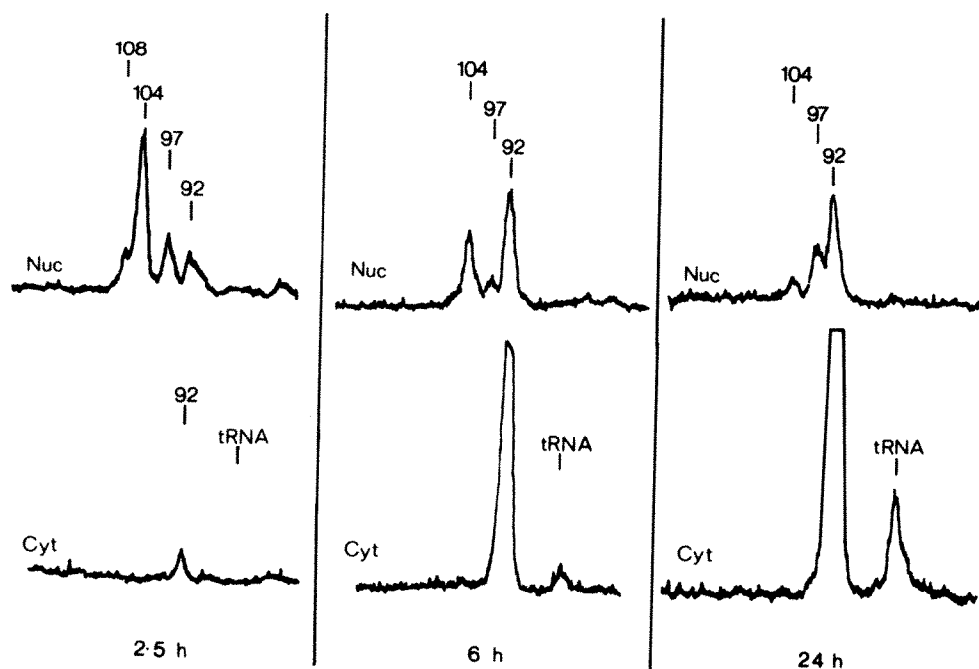


Fig. 2 Intracellular location of $tDNA^{tyr}$ transcripts. Single oocytes which had been previously injected with $tDNA^{tyr}$ and [α - ^{32}P]GTP were manually dissected⁴² after incubation at 19 °C for 2.5, 6 and 24 h and the RNAs present in each individual nucleus and cytoplasm were electrophoresed in 10% acrylamide gels¹⁸. The autoradiograms of these gels (like that in Fig. 1) were traced with a Joyce-Lobel densitometer. The tracings from three representative oocytes are shown. Nuc, nucleus; Cyt, cytoplasm.

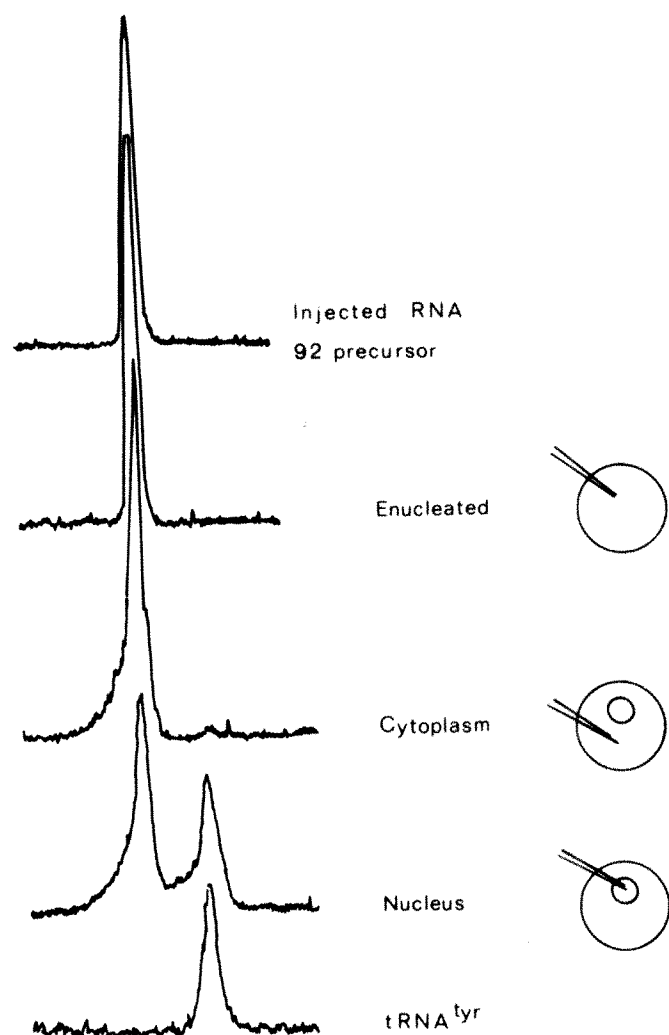


Fig. 3 Splicing of the intervening sequence is associated with the nucleus. Radioactive 92-base $tRNA^{tyr}$ precursor from injected oocytes was purified by extraction from acrylamide gels²⁵. This ^{32}P -labelled precursor, the substrate for the splicing enzyme(s), was injected into enucleated oocytes⁴³, the cytoplasm, or the nucleus of intact oocytes using approximately 5,000 d.p.m. of ^{32}P -labelled RNA per oocyte. After an 18-h incubation the injected oocytes were homogenised and the ^{32}P RNAs were extracted and electrophoresed in a 10% acrylamide gel. Two uninjected markers, for the 92-base precursor and the spliced $tRNA^{tyr}$, were electrophoresed in adjacent tracks. The densitometer tracings of this acrylamide gel are shown.

$tDNA^{25}$ and yeast serine $tDNA$ (J. Broach, R. Cortese and D. A. Melton, unpublished data). In all these cases, the common feature of the $tRNA$ precursors which are partitioned in the nucleus is that they have unfinished termini, that is, they have 5' leader and 3' trailer sequences.

Splicing of the intervening sequence occurs in the nucleus

A splicing activity has been isolated from nuclear preparations⁶ and from a cytoplasmic ribosomal wash in yeast⁸. However, the difficulties in obtaining pure preparations of nuclei and cytoplasmic fractions have led to uncertainties about the location of the splicing enzymes. Our results based on the dissection of injected oocytes (Fig. 2) show a similar ambiguity in that the 92-base precursor, the substrate for the splicing enzyme, is found in both the nucleus and cytoplasm whereas the mature $tRNA^{tyr}$ is found mostly in the cytoplasm. The two obvious possibilities for the removal of the intervening sequence are that

(1) the 92-base precursor is transported to the cytoplasm and then the intervening sequence is removed to give mature $tRNA^{tyr}$ or (2) the splicing occurs in the nucleus and the mature $tRNA^{tyr}$ is then quickly (or even as a consequence) transported to the cytoplasm.

We have tested the location of the splicing enzyme(s) in intact cells by injecting ^{32}P -labelled 92-base precursor RNA into the nucleus, the cytoplasm, and into enucleated oocytes. Figure 3 shows that a significant amount of the 92-base precursor is spliced only when the RNA is injected into the nucleus. This strongly suggests that the last step in tailoring the size of the precursor, the excision of the intervening sequence and subsequent ligation of the two $tRNA$ halves, is a nuclear event. Therefore, the splicing enzyme or enzyme complex might be in the nucleoplasm or located at the nuclear membrane. This conclusion is not affected by the problems posed by the biochemical purification of nuclei.

The splicing step is inefficient in injected oocytes: even after 6 d of $tDNA^{tyr}$ transcription only 30% of all the transcripts are spliced into mature $tRNA^{tyr}$ (ref. 12 and Fig. 2). The nuclear location of the splicing enzyme(s) could explain this inefficiency. Once the 92-base precursor enters the cytoplasm it is probably no longer accessible to the splicing enzyme(s). The 'leakage' of the 92-base precursor into the cytoplasm which we observe may result from the fact that the oocyte is overloaded with the transcripts of a single gene. One would not expect this to occur in normal yeast cells.

Post-transcriptional addition of CCA

All transfer RNAs have the sequence CCA at their 3' end, the attachment site for the amino acid. These three bases are not encoded in the yeast $tDNA^{tyr}$ and must therefore be added post-transcriptionally⁵.

The results presented in Fig. 4 show that the CCA is not present on the 104-base precursor. Analysis of the 97-base precursor (not shown) reveals that it has the same 3' terminus as the 104-base precursor, the CCA still being absent. By the 92-base precursor stage all of the molecules have a 3' CCA (Fig. 4f).

The presence of the CCA on the nuclear 92-base precursor suggests that these bases are added to the newly synthesised RNA in the nucleus. However, it seemed likely that the CCA-adding enzyme ($tRNA$ nucleotidyl transferase) would also be found in the cytoplasm because it is known that the 3' CCAs of $tRNAs$ are constantly removed and replaced or repaired in the cell^{26,27}. This was tested directly by injecting deadenylated $tRNA$ into different cell compartments. Deadenylated $tRNA$, a substrate for $tRNA$ nucleotidyl transferase, was prepared by chemically removing the 3'-terminal A residue from purified $tRNA^{tyr}$ (ref. 28). The results in Fig. 5 show that a 3'-terminal A residue can be added to deadenylated $tRNA^{tyr}$ in the cytoplasm as well as in the nucleus. Taken together with the results shown in Fig. 4, this shows that the enzyme $tRNA$ nucleotidyl transferase is present in both the nucleus and cytoplasm of the cell.

The experiments described so far suggest the following order for the size alterations of the $tRNA^{tyr}$ precursor: (1) stepwise removal of the 5' leader and 3' trailer sequences, (2) addition of a 3'-terminal CCA and (3) splicing of the intervening sequence. All of these processing events occur in the cell nucleus. In addition to these processing steps, the maturation of a $tRNA$ molecule involves modification of specific ribonucleotides. The relationship between the size alterations and base modifications of the $tRNA^{tyr}$ precursors is described below.

Ribonucleotide modifications occur in an obligatory order

The intracellular site at which the ribonucleotides are modified was examined by analysing the RNA fingerprints of the nuclear and cytoplasmic $tRNA^{tyr}$ precursors. The T_1 ribonuclease fingerprints of the nuclear 104-base, nuclear 92-base and cytoplasmic 92-base precursors are shown in Fig. 4. The minor bases

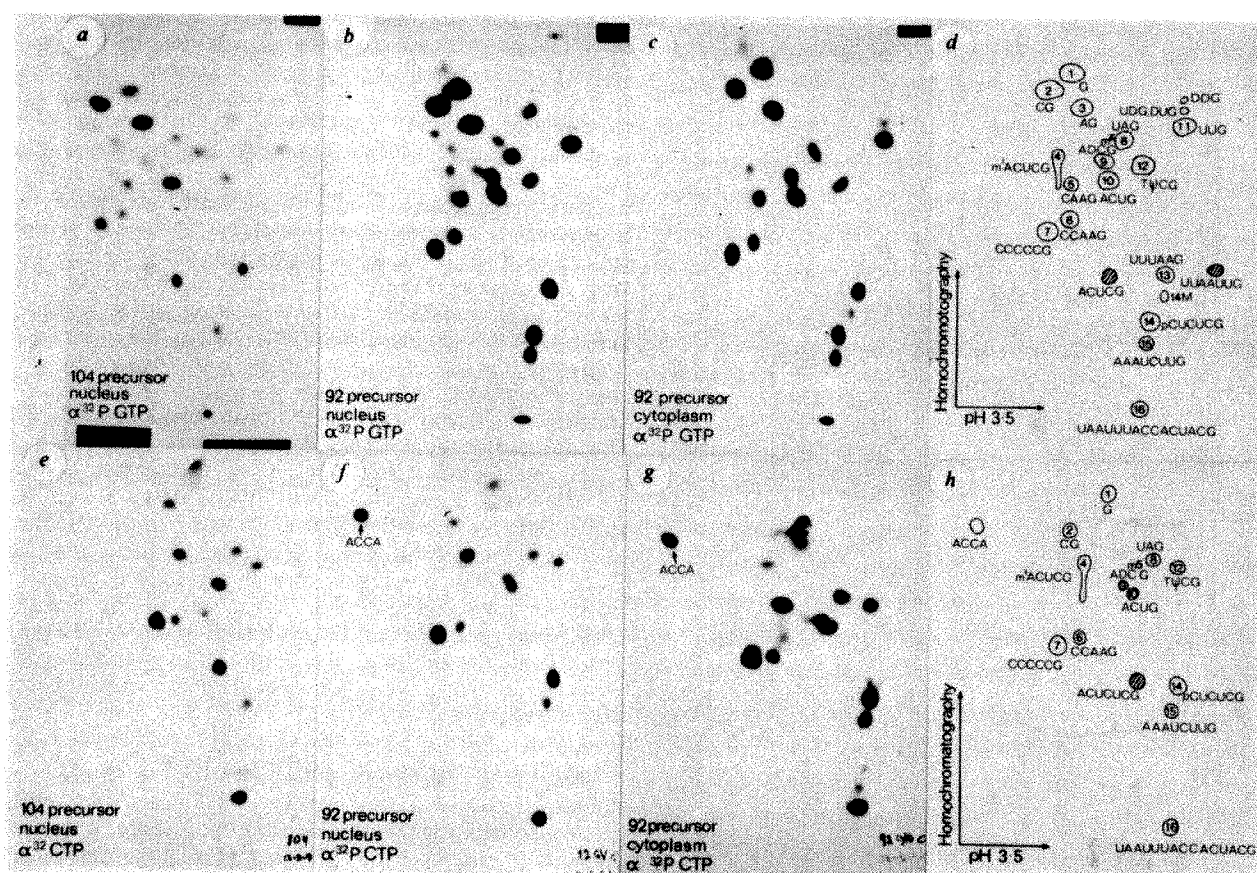


Fig. 4 RNA fingerprints of tRNA^{tyr} precursors synthesised in injected oocytes. ³²P precursor RNAs were isolated either from the nucleus or the cytoplasm of oocytes 5 h after injection with tDNA^{tyr} and [α-³²P]GTP (a–d) or [α-³²P]CTP (e–h). Following digestion with ribonuclease T₁ the oligonucleotides were separated in one dimension on cellulose-acetate at pH 3.5 and in the second dimension by homochromatography on DEAE-cellulose thin layer plates⁴⁴. Some background of small oligonucleotides from endogenous oocyte transcripts is apparent in the upper part of panels a and b. This is because the RNA bands analysed were relatively weak and the fingerprints had to be exposed for long periods. The diagrams at the right indicate the composition of the oligonucleotides; hatched spots are oligonucleotides unique to the 104-base precursor. For the numbering of the T₁ oligonucleotides and their position within the tRNA^{tyr} precursor see refs 5 and 12. Note the 3'-terminal CCA is present in the 92-base precursors (f, g), but absent in the 104-base precursor (e). Some base modifications affect the mobility of certain oligonucleotides, for example D in oligonucleotide 9, as discussed in the text.

present in the precursors were analysed by elution of the T₁ oligonucleotides, hydrolysis with alkali and thin layer chromatography²⁹. Table 1 summarises the results of this type of nearest neighbour analysis for some selected T₁ oligonucleotides, and shows that most of the base modifications analysed, including 5-methyl-cytosine (m⁵C), 1-methyladenosine (m¹A), pseudouridine (Ψ), and dihydrouridine (D), occur in the nucleus. The only modification found to be exclusively cytoplasmic is a G modification in T₁ oligonucleotide 14. This uncharacterised modification (see Table 1) causes the appearance of a new spot in the RNA fingerprint of the cytoplasmic 92-base precursor (see Fig. 4c, oligonucleotide 14M). It should be noted that although this nucleotide is not normally modified in yeast, all the other modifications described in Table 1 are normally present in yeast tRNA^{tyr}.

Analysis of the RNA fingerprints of the various tRNA^{tyr} precursors (as in Fig. 4) also showed that base modifications occur in an obligatory order which is strictly correlated with the size alterations of the tRNA precursors. Some modifications are present on precursors which have 5' leader and 3' trailer sequences while others do not occur until these sequences are removed (Table 1).

The correlation between the size reduction and base modification of the precursors is easily demonstrated in the cases where the base modifications cause a change in the mobility of a T₁ oligonucleotide. For example, oligonucleotide 9, which has the sequence ADm⁵CG, can be resolved from oligonucleotide 10, ACUG, only when the dihydrouridine (D) is present. In the 104-base precursor the two oligonucleotides, 9 and 10, overlap

(Fig. 4a) whereas in the 92-base precursor, which contains D in oligonucleotide 9, the two spots are separated in the fingerprint (compare Fig. 4a with 4b and c). This particular modification, U→D, is observed only in precursors which have had the 5' leader removed and the 3' CCA added. Other modifications, as indicated in Table 1, are found on precursors containing 5' leader and 3' trailer sequences. In the case of oligonucleotide 4, m¹ACUCG, the methylation of the adenosine causes an increased mobility in the second dimension of the fingerprint analysis. This nucleotide is already completely modified to m¹A at the 104-base precursor stage (Fig. 4e).

Perhaps the most striking example of the order in tRNA processing is provided by the case of two pseudouridine (Ψ) modifications. In the 104-base precursor there is a Ψ in the TΨC loop (oligonucleotide 12), but the Ψ which will eventually appear in the anticodon stem (oligonucleotide 15) is absent. Once the 5' leader sequence is removed and the 3' CCA added, the U in the anticodon stem of the 92-base precursor is completely modified to Ψ (Table 1). It should be noted that only a selected subset of the possible base modifications was analysed here. Other base modifications may well occur.

We therefore observe an apparently invariant order in the processing of the tRNA^{tyr} precursors. The order of events is said to be invariant in that we do not find, for example, precursors which have had the intervening sequence removed but still retain a 5' leader sequence. Nor, for example, are certain nucleotides modified in precursors which still have a 5' leader. Conventionally, the maturation of tRNA precursors is divided into two separate processes: size reduction and base

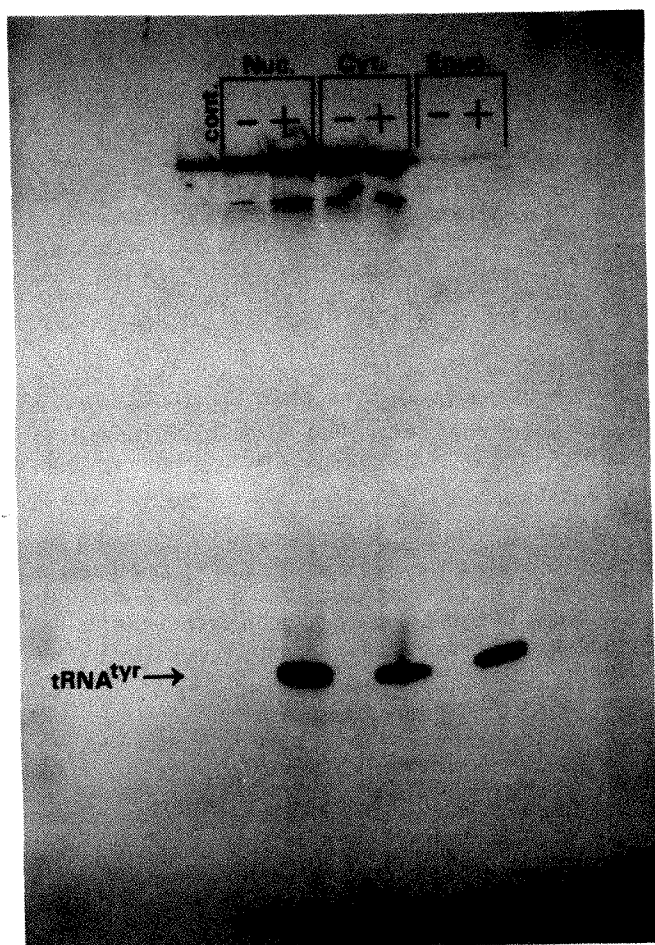


Fig. 5 Addition of a 3'-terminal A residue to transfer RNA in injected oocytes. [α - 32 P]ATP (10 mCi ml $^{-1}$) was injected with (+) or without (–) deadenylated tRNA^{tyr} (25 ng per oocyte) into either the nucleus (Nuc) or the cytoplasm (Cyt) of intact oocytes and into enucleated oocytes (ENUC). After an incubation of 1.5 h the RNA was extracted and electrophoresed in a 10% acrylamide gel as in Fig. 1. Pure tRNA^{tyr} (provided by M. Olson) had the 3'-terminal A removed by periodate treatment²⁸. Pure tRNA^{tyr} which had not been treated with periodate was injected into oocytes with [α - 32 P]ATP as the control (cont.).

modification. The results presented here show that these processes are in fact intimately related and proceed in concert with each other.

Discussion

A plausible scheme for the steps involved in the biosynthesis of yeast tRNA^{tyr} as observed in injected oocytes is shown in Fig. 6. Perhaps the most striking aspect of this pathway is that almost all of the RNA processing occurs in the nucleus. From the nuclear location of the tRNA^{tyr} precursors one can infer that certain maturation enzymes must be located in the nucleus. These include the enzymes that synthesise 1-methyl-adenosine, 5-methyl-cytosine, pseudouridine, and dihydrouridine (Table 1). Furthermore, experimental evidence has been presented to show that the removal of the 5' leader sequence, splicing, and addition of the 3' CCA all occur in the nucleus (Figs 3, 5, ref. 25). Earlier studies based on the biochemical fractionation of nuclei and cytoplasm indicated that tRNA precursors are found and processed in the cytoplasm^{26,30–33}. With different methods, namely the manual isolation of the oocyte's large nucleus and the direct injection of radioactive RNAs into various cell compartments, the results reported here and those of Lönn³⁴ support the conclusion that almost all of the maturation of tRNA precursors occurs in the nucleus.

Table 1 Order and intracellular location of base modifications

Ribonucleotide modification	T ₁ oligonucleotide	Precursor (bases)		
		104 nucleus	92 nucleus	92 cytoplasm
m ¹ A	4 (TΨC loop)	+	+	+
m ⁵ C	9 (variable loop)	+	+	+
Ψ	12 (TΨC loop)	+	+	+
	15 (anticodon stem)	–	+	+
D	9 (variable loop)	–	+	+
	11 (D loop)	–	+	+
G*	14M (aa stem)	–	–	+

The 104-base and 92-base tRNA^{tyr} precursors were isolated from either the nucleus or cytoplasm of oocytes which had been injected with tDNA^{tyr} and [α - 32 P]GTP or [α - 32 P]CTP. The modified bases present in selected T₁ oligonucleotides were characterised by pancreatic RNase digestion or alkali hydrolysis followed by cellulose thin layer chromatography in isopropanol: H₂O:HCl (70:15:15 v/v)⁴⁵ or paper electrophoresis at pH 3.5 (ref. 44). The T₁ oligonucleotides are numbered as in Fig. 4. Note that most of the modifications occur in the nucleus (except for G*) and that some modifications only occur after the termini of the precursor have been matured. m¹A, 1-methyladenosine; m⁵C, 5-methylcytosine; Ψ, pseudouridine; D, dihydrouridine; G* is an uncharacterised G modification whose migration by paper electrophoresis is consistent with it being Gm (2-O-methylguanosine) or some other modification that renders G resistant to ribonuclease T₁ cleavage.

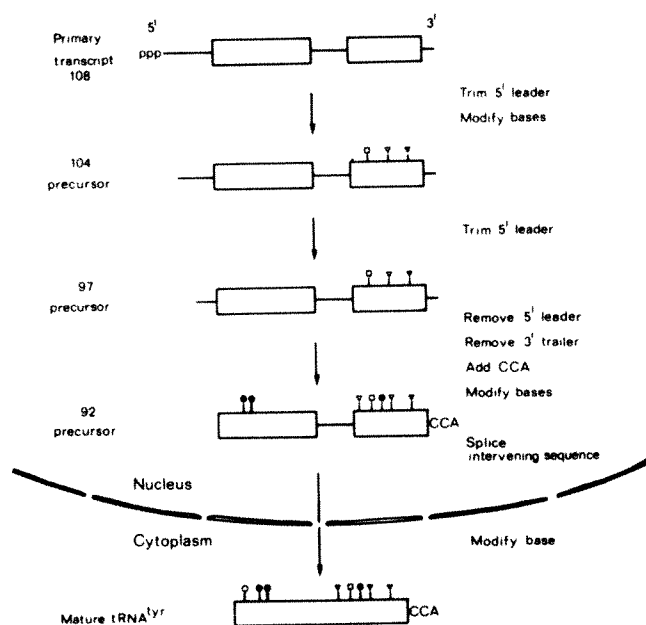


Fig. 6 RNA processing of tRNA^{tyr} precursors into mature tRNA^{tyr} in injected oocytes. The primary transcript of tDNA^{tyr} is a precursor 108(±4) nucleotides in length with a 5' leader, intervening sequence and 3' trailer (as in Fig. 1). This is the only precursor which has a 5'-terminal triphosphate¹². The 5' leader is removed in three stages, the last of which is accompanied by excision of the 3' trailer and addition of the 3' CCA. The position and type of the base modifications are indicated as well as the stage at which they appear. The modified bases in the 108-base and 97-base precursors have not been analysed. Therefore some of the modified bases shown to be newly added at the 92-base precursor stage may be present on the 97-base precursor, but these are definitely absent in the 104-base precursor (Table 1). Note that only some base modifications have been analysed; others may well occur. The 92-base precursor which enters the cytoplasm (Fig. 2) is not shown because our results suggest that the cytoplasmic 92-base precursor is not processed to mature tRNA (see text). □, 5-methyl-cytosine; ▽, pseudouridine; ▼, 1-methyladenosine; ●, dihydrouridine; ○, G*, an uncharacterised G modification.

Superimposed on the spatial order of the tRNA processing we have observed a correlation between the size changes and base modifications of the tRNA precursors. Previous studies have suggested that the length reduction of tRNA precursors occurs in a precise order^{4,35-39}, but little work has been done on its connection to base modification. Two notable exceptions from studies on bacterial tRNAs are the demonstration that a Gm modification is absent in a bacteriophage T₄ tRNA precursor⁴⁰ and *in vitro* studies on the effect of pseudouridine modifications on *E. coli* tRNA^{tyr} cleavage by RNase P (ref. 41). The obligatory order observed for the yeast tRNA^{tyr} base modifications can be explained by assuming that each of the tRNA^{tyr} precursors is a substrate for a particular subset of maturation enzymes. For example, the presence of a 5' leader sequence could introduce order into the maturation process by altering the tertiary structure of the precursor. Some maturation enzymes might recognise precursors with a 5' leader while others would accept the precursor as a substrate only after the 5' leader has been removed. The specific sequence in which the bases of the 104-base precursor and then the 92-base precursor are modified provides some support for this suggestion (Fig. 6). The possibility remains that the intervening sequence might similarly order an additional round of base modifications, but this was not tested in the present study.

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In conclusion, it is not immediately obvious why a gene product as apparently simple as a tRNA is synthesised via such a complicated processing scheme. One hint, however, comes from our previous observation¹² that different genetic loci of the same tRNA gene are transcribed into RNA precursors all of which have a 5' leader, though the length and sequence of this extra segment varies in each case. The fact that the 5' leader is present in the case of the four yeast tyrosine tDNAs and the two yeast serine tDNAs examined suggests that it may have some biological function (ref. 12 and J. Broach, R. Cortese and D. A. Melton, unpublished data). Our studies implicate the 5' leader as having a possible role in two related aspects of tRNA processing: the ordering of base modifications and the nuclear segregation of tRNA precursors. Together these results suggest that a possible function for extra terminal sequences on tRNA precursors is to ensure that normally only a mature tRNA is presented to the cytoplasm for participation in protein synthesis.

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LETTERS

IUE observations of the hot components in two symbiotic stars

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Recent IUE observations reveal striking differences in the UV spectra of two symbiotic stars, R Aqr and RW Hya. RW Hya is found to be an unexpectedly intense source of UV radiation. The measurements reported here demonstrate the presence of a hot component in each star, supporting the view that each is a binary system with a luminous red primary and a hot, sub-luminous companion. In one case, the hot companion manifests itself by exciting a compact nebulosity; in the other case we believe that the continuous spectrum of the hot star is directly detected, while the continuum of nebulosity excited by the hot star is detected at longer wavelengths.

Our observations were made in the wavelength range 1,200-3,200 Å. The UV continuum found in the spectrum of R Aqr and shown in Fig. 1a is most easily interpreted as emission from a dense and compact nebula, which must be ionised by UV photons from a hot secondary. From visible light spectra, R Aqr is classified¹ as M7+pec. The continuum seen in Fig. 1a is ascribed to Balmer emission that arises from recombination radiation of hydrogen atoms. The nebula also produces both permitted and semi-forbidden lines of various ions. Observations in the IUE long-wavelength range (1,900-3,200 Å) also show the forbidden line of O II at λ 2,470 Å and a strong emission feature at λ 2,328 Å that must represent a blend of λ 2,321 Å [O III] and λλ 2,325, 2,327, 2,328 Å C II]. The most prominent emission lines include C III], C IV, Si II, Si III] and Si IV (Fig. 1a). Lower intensity features are not indicated since blends due to the ~6 Å spectral resolution make some identifications of weak lines ambiguous. The λλ 1,808 and 1,806 Å lines of Si II, for example, most likely correspond to the broad emission feature evident in Fig. 1a.

The relatively low excitation spectrum of the nebula indicates that the nebula is excited by a star (with effective temperature $T \geq 50,000$ K) that resembles a white dwarf or the central star of a planetary nebula. Only such a star can produce enough

ionising photons as required by the present measurements ($\sim 10^{42} \text{ s}^{-1}$) and yet be sufficiently faint (given the estimated distance of $\sim 260 \text{ pc}$) (ref. 1) so that the stellar continuum does not dominate the UV spectrum of R Aqr. Our computations are based on an estimated colour excess, $E(B-V) \approx 0.05 \text{ mag}$, corresponding to an interstellar extinction at wavelength $1,550 \text{ \AA}$, $A_\lambda \approx 0.37 \text{ mag}$.

The feature at $\sim 1,250 \text{ \AA}$ is indicated in Fig. 1 as composite emission of S II and N V. However, presumably it is dominated by S II as the relatively low excitation of this ion is consistent with the nebular temperatures deduced from the oxygen and carbon line intensities. Our low spectral resolution observations cannot positively differentiate one ion species from another in this feature. However, we find that a compact dense nebula of $n_e \sim 10^6 \text{ cm}^{-3}$ and temperature $T \geq 15,000 \text{ K}$ suggests that it is due mainly to S II rather than the higher excitation emission line of N V. Also, the UV emission observed is not adequately explained by a hot chromosphere in the M giant primary, because the densities required to produce other emission lines observed ($n_e \sim 10^7\text{--}10^8 \text{ cm}^{-3}$) at typical chromospheric temperatures $T \leq 10,000 \text{ K}$ at these densities would be inconsistent with the presence of [O III] emission at $2,321 \text{ \AA}$ that is observed in the IUE long-wavelength range.

RW Hya is classified gM2 + pec and varies between 10 and 11 visual magnitudes. Attributing the visual flux to the red giant primary, we estimate the distance to the system as $\sim 1 \text{ kpc}$. Although the presence of hot companions in symbiotic stars has been suspected, the intensity of UV radiation from RW Hya is surprising. The resonance lines of the C IV ion at wavelengths $1,548, 1,550 \text{ \AA}$ are so bright that they saturate in a single pixel in an exposure time of 30 s with the short wavelength, low dispersion IUE spectrometer. The star appears to be essentially unreddened and is notable for the strong UV continuum that (Fig. 1b) rises towards short wavelengths. The distance of $\sim 1 \text{ kpc}$ deduced for RW Hya was obtained from the interstellar absorption of Ly α that produces an absorption equivalent width $w_{\lambda 1,216} \sim 1.5 \text{ \AA}$. The corresponding hydrogen column density in our line of sight $N_{\text{H I}} \sim 6.4 \times 10^{18} \text{ cm}^{-2}$ corresponds to an extinction $E(B-V) \approx 0.001$. This small absorption value is attributed to the height of RW Hya above the galactic plane ($l = 316^\circ$, $b = +36.5^\circ$) which places the star at a distance $\sim 700 \text{ pc}$ above the plane of the galaxy.

The continuum observed in RW Hya fits a Rayleigh-Jeans approximation to a black-body law that corresponds to an effective temperature $T \geq 10^5 \text{ K}$, with a peak that probably occurs well below the wavelength range of IUE near 500 \AA . Model atmospheres for hot white dwarfs show that in the UV range over which we observe a strong continuum, the thermal emission of such stars is approximated closely by that of a black body⁵. This must represent the emission of a subluminescent star, brighter and hotter than the companion of R Aqr. The continuum of the hot component in RW Hya, if it continues to follow the Rayleigh-Jeans law, must fall well below the continuum that we actually observe above $1,900 \text{ \AA}$ with the long wavelength, low dispersion IUE spectrograph. The long wavelength UV continuum, much too bright to arise in the M primary, is ascribed to nebulosity excited by the secondary star. Although both permitted and semi-forbidden emission lines are found, we have not identified any forbidden line in the UV spectrum of RW Hya, suggesting that the nebulosity in this star may be more dense (and perhaps smaller) than that in the R Aqr system.

The physical conditions that are responsible for the UV emission from these two symbiotic stars are notably different. The relatively low excitation emission observed in the line spectrum of R Aqr is indicative of a nebula photoionised by the hot star in the system. The prevailing density in the nebula, as deduced from the carbon and forbidden oxygen line strengths, must be roughly 10^6 cm^{-3} , while the scale of the nebula is $\leq 10^{15} \text{ cm}$ and the electron temperature is $\approx 15,000 \text{ K}$. The electron density and temperature of the nebula in which the primary and secondary are embedded was obtained from the

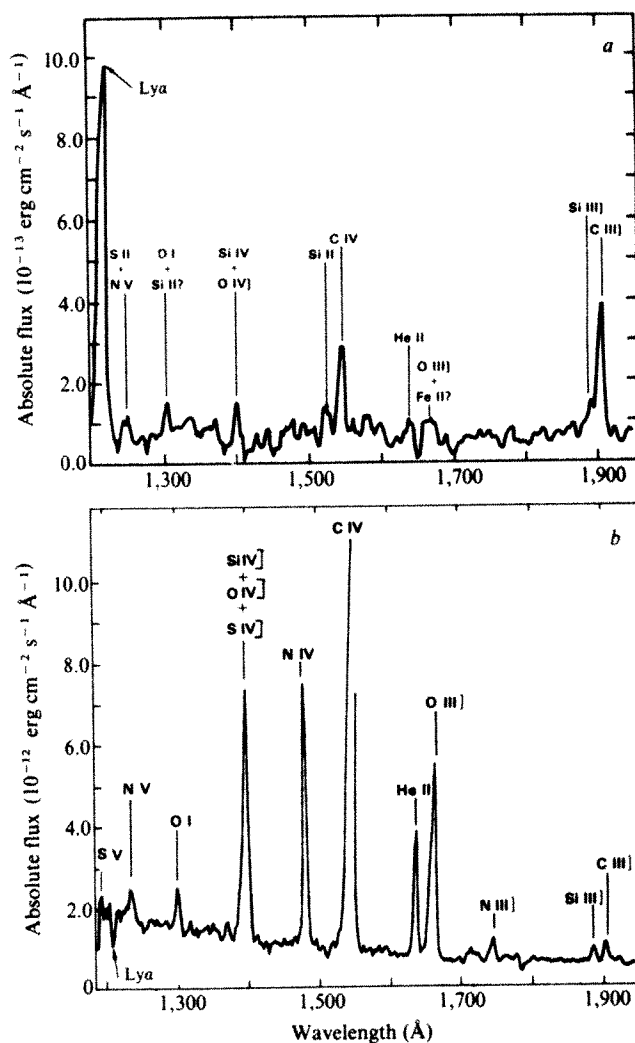


Fig. 1 UV spectra of the symbiotic stars R Aqr (a) and RW Hya (b) obtained with the short wavelength, low dispersion spectrograph of the IUE observatory satellite on 29 July 1979 and 1 September 1979 respectively. Note in b that the resonance lines of C IV at $1,548 \text{ \AA}$ and $1,550 \text{ \AA}$ are off scale and that the pronounced continuum rises towards shorter wavelengths, and that Ly α is in absorption. The spectra have been corrected for the absolute response of the instrument, but not for interstellar reddening, which affects the R Aqr spectrum. The Ly α emission line observed in R Aqr represents combined geocoronal and stellar emission.

relation and parameters of Osterbrock² for planetary nebulae, where we have used appropriate cosmic abundances for oxygen and carbon. Here the observed flux for a particular emission line is assumed to be proportional to $n_e^2 L^3$ for a given temperature, distance and cosmic abundance. Note that the scale size L of the nebula is comparable to the diameter of the hot star's orbit, if the orbital period is $\sim 27 \text{ yr}$ (ref. 3).

In contrast, the lack of significant emission in the $2,321 \text{ \AA}$ line of [O III], and the strong permitted line emissions, suggest that the nebulosity in RW Hya is much denser, $\sim 10^9 \text{ cm}^{-3}$, and hotter, $T_e = 20,000 \text{ K}$. The strong permitted line emissions, such as those due to C IV and O I, may be the result of mechanical heating of the gas as the result of tidal interaction between the extended envelope of the red giant primary and the compact companion. Mass accretion onto the surface of the companion can result in thermal heating of the accreting surface⁶. The He II line at $1,640 \text{ \AA}$ observed in both symbiotic stars is observed to be far more intense in RW Hya than R Aqr, confirming that RW Hya is a higher excitation source. The relatively weaker He II $1,640 \text{ \AA}$ emission observed in the spectrum of R Aqr probably reflects the lower ionisation of a nebula that is excited by a relatively cooler companion star. The presence of this high temperature line is possibly explained by emission arising in very

close proximity to the hot component with coronal temperatures.

Mass transfer may be a significant energy source in RW Hya and may be related to the intense UV emission and to the higher apparent effective temperature of its companion star, as compared with R Aqr. As in other types of binary systems, a distinguishing characteristic among individual symbiotic stars may be the degree of mass transfer onto the compact star. This is supported by IUE observations of CH Cyg, which reveal an UV continuum ascribed to mass transfer from the M6–M7 III primary on to the hot subluminescent secondary which results in an optically thick UV-emitting corona around the secondary⁶, a model supported by optical spectra as well. Given the energetics of the accretion process in white dwarfs, luminosities of $\sim 10^{36}$ erg s⁻¹ in the X-ray regime (1–4 keV) seem possible⁴. Accordingly, RW Hya may be a detectable source with the imaging proportional counter on the HEAO 2 (Einstein) observatory.

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A model for ball lightning

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An eyewitness sees ball lightning as a luminous sphere in regions of thunderstorm activity. There are many reports of ball lightning forming after a lightning discharge, existing for several seconds, and terminating suddenly and silently, or exploding with or without material damage^{1,2}. Jennison³ has described ball lightning observed at close range inside a commercial aircraft shortly after a lightning discharge. As it drifted down the aisle of the passenger cabin, this ball lightning exhibited: (1) perfect equilibrium in a perfectly spherical shape with diameter 22 ± 2 cm; (2) 5–10 W of optical radiation in blue-white light, but no heat radiation; and (3) an optically thick surface of almost solid appearance, devoid of polar or toroidal structure, but with some limb-darkening. It seems unreasonable to dismiss Jennison's ball lightning as an optical illusion⁴ (afterimage of the eye's retina). A physical explanation in terms of a microwave cavity⁵, a d.c. glow discharge⁶, chemical reactions⁷, nuclear reactions⁸ or annihilation of antimatter⁹ runs into difficulties since the all-metal airplane structure would act effectively as an air-tight Faraday cage. A plethora of excited atoms and molecules, and electromagnetic waves ranging from radio frequencies to X rays are produced in the channel of a lightning discharge, but only electric charge itself can enter the interior of an all-metal airplane without difficulty. Here a new model is presented which explains ball lightning in terms of electric charge.

Charge transfer through ionised air generally obeys Ohm's law $\mathbf{j} = \sigma \mathbf{E}$, where \mathbf{j} stands for current density and \mathbf{E} for electric field. The scalar factor σ is the electrical conductivity. The discharge current magnetises the ionised region where it passes. This magnetisation is dissipated into heat on a time scale known as the magnetic diffusion time τ . Magnetic field lines are 'frozen into' the plasma for a time span¹⁰:

$$\tau = \mu \sigma L^2 \quad (1)$$

where μ is magnetic permeability, σ is electrical conductivity and L is a typical length scale of the ionised region. From his measurements of the emission line profile of H α , Uman¹¹ has calculated a conductivity of 1.8×10^{14} S m⁻¹ during the high-temperature phase of a lightning discharge. With vacuum permeability $\mu_0 = 4\pi \times 10^{-7}$ N/A², and with 10 cm diameter for the lightning channel, equation (1) yields a magnetic diffusion time of 2.3×10^{-4} s. This short time scale is clearly incompatible with observed ball lightning lifetimes, but it can easily account for instantaneous disappearance of a magnetised plasma sphere in air.

Equation (1) offers two ways of suppressing magnetic dissipation by ohmic losses in the plasma. First, the magnetic permeability μ could be larger than the vacuum permeability μ_0 , as in ferromagnetic materials. Below the Curie temperature, a ferromagnet has a permanent magnetic field, corresponding to $\mu = \infty$ in equation (1). Second, the electrical conductivity σ could be higher than in the lightning channel. Infinite conductivity is found in superconducting materials below the transition temperature, where electrical currents persist indefinitely. Uman's lightning channel conductivity of 1.8×10^{14} S m⁻¹ holds good for laminar plasma motion only. Plasma turbulence will enhance charge transfer by translations of charged fluid parcels, and rotations of charged fluid parcels will generate a magnetic field. Our first hypothesis is that turbulence in the ball lightning plasma enhances charge transfer to the point that charged vortices transport electricity the way electrons do in a superconductor. By this hypothesis, individual plasma vortices have a strong tendency to travel with the same momentum and spin with aligned axes; as such they behave macroscopically the way bosons do on the atomic level.

Feynman¹² has shown that a fluid consisting of charged bosons has potential energy of the form $\nabla^2 \rho^{1/2} / \rho^{1/2}$, where ρ stands for the charge density, or, by virtue of Coulomb forces between electrons and ions, for mass density. This potential energy is zero for a uniform distribution ρ is constant (as in solids), but it can be positive as well as negative in a density-stratified plasma with non-uniform ρ . Correcting the sign of Feynman's expression we write $U = -C \nabla^2 \rho^{1/2} / \rho^{1/2}$ ($C > 0$) for the potential energy U , corresponding to interaction between plasma vortices with a force field $-\nabla U = \nabla C \nabla^2 \rho^{1/2} / \rho^{1/2}$. In hydrostatic equilibrium this force field is balanced by pressure forces only, hence¹⁶:

$$0 = \frac{-1}{\rho} \nabla p + \nabla C \frac{\nabla^2 \rho^{1/2}}{\rho^{1/2}} \quad (2)$$

where the total pressure p includes kinetic pressure from electrons and ions, and turbulent pressure from plasma vortices.

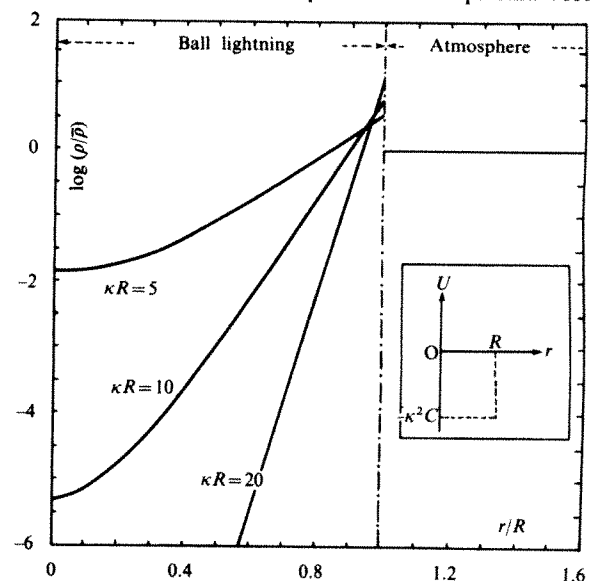


Fig. 1 Three density profiles of isobaric ball lightning models with different binding energies. Inset, square well potential energy of isobaric density profiles.

Each plasma vortex exerts centrifugal forces on its neighbours, which dominate over kinetic pressure when plasma circulation inside the vortex exceeds the local speed of sound. Such supersonic circulation causes density gradients on the length scale of one plasma vortex, resulting in a sponge-like structure of tenuous vortex cores nested in dense ambient plasma.

Collective motions of electrons and ions on length scales smaller than one Debye length are dissipated by Landau damping¹⁵, and local deviations from charge neutrality are screened out on length scales larger than one Debye length¹⁵. Thus the sizes of plasma vortices behaving as charged bosons apparently lies around one Debye length. Since Debye length scales as $T^{1/2}/n^{1/2}$ with temperature T and electron density n , a hot and tenuous region of the ball lightning plasma has larger vortices with more electrons and ions than a cooler and more dense region. High-pressure arc plasmas typically have a Debye length around 10^{-6} cm, which may serve as a lower limit on the size of plasma vortices in our ball lightning model.

The virial theorem⁶ yields $3pV$ as an upper limit on the energy content of a plasmoid with pressure p and volume V . Confinement by atmospheric pressure only, results in a maximum energy content of $\sim 10^3$ J, which is several orders of magnitude below ball lightning energies reported elsewhere^{1,8}. Some ball lightning theories avoid this difficulty by postulating a continuous energy input through radiowaves⁵ or d.c. currents⁹. Our boson model for plasma turbulence lifts the virial constraint on maximum energy content with negative potential energy from interaction between plasma vortices. With internal confinement forces as in equation (2), hydrostatic equilibrium of the ball lightning plasma demands internal pressures above 1 atm. The energy $\int p dV$ stored in the pressure distribution includes thermal energy from electrons and ions, and turbulent energy from vortex motion.

Equation (2) is nonlinear except in the isobaric case where p is constant. For spherical symmetry, the non-singular isobaric solution with negative potential energy $U = -\kappa^2 C$ is^{13,16}:

$$\rho^{1/2} = \rho_C^{1/2} \frac{\sinh(\kappa r)}{\kappa r} \quad (3)$$

where ρ_C denotes the central density of the plasma sphere, and $1/\kappa$ is a length scale. In the limit $\kappa \rightarrow 0$, equation (3) represents a uniform and isothermal plasma sphere without potential energy. Density stratification appears as κ increases from zero, giving progressively more negative potential energies $-\kappa^2 C$, and lower central densities ρ_C . A complementary stratification in temperature maintains isobaric conditions in the gas. Thus equation (3) represents a plasma sphere with a hot and tenuous central region surrounded by a cooler and more dense 'gas blanket'.

For a ball lightning floating in air, the mean density $\bar{\rho}$ cannot be far from atmospheric density, 1.3 kg m^{-3} . On squaring and integrating equation (3) over the volume of a sphere with radius R , the mean density comes out as $\bar{\rho} = 3\rho_C \{[\sinh(\kappa R) \cosh(\kappa R) - \kappa R]/2\kappa^3 R^3\}$. Using $\sinh(\kappa R) = \cosh(\kappa R) = \frac{1}{2}e^{\kappa R} \gg \kappa R$ for $\kappa R \gg 1$, the ratio of central density ρ_C and surface density ρ_s to mean density $\bar{\rho}$ is approximately:

$$\frac{\rho_C}{\bar{\rho}} \approx \frac{8}{3} \kappa^3 R^3 e^{-2\kappa R}; \quad \frac{\rho_s}{\bar{\rho}} \approx \frac{2}{3} \kappa R \quad (4)$$

which hold to better than 1% for $\kappa R \geq 5$. Density profiles relative to mean density are sketched in Fig. 1 for $\kappa R = 5, 10$ and 20. At the centre $r = 0$, all profiles are horizontal, as required for a physically acceptable solution. By assumption, the whole interior region $r < R$ is isobaric and therefore force-free. Binding forces from negative potential energy only appear as stresses on the surface $r = R$, where the density abruptly returns to atmospheric conditions over a small distance Δr . In the limit $\Delta r \rightarrow 0$, this discontinuity in ρ results in surface stresses tending to infinity as $1/(\Delta r)^3$, by equation (2). A pressure discontinuity maintains hydrostatic equilibrium at $r = R$. Inasmuch surface temperature as well as surface density are above atmospheric

conditions, interior pressures must be above 1 atmosphere. Low- κ solutions with little excess pressure should represent a low-energy ball lightning with restricted radiative output, as seen by Jennison. Other ball lightning reports specify internal energies in the range 10^6 – 10^8 (ref. 8), and a scorching radiative output of $4.5 \times 10^5 \text{ W}$ (ref. 14). This type should correspond to the high- κ limit of equation (4), where severe density stratification and high surface temperatures imply huge internal pressures. Already for moderate values of κ , the central density and temperature calculated from equation (4) fall in the region of thermonuclear interest. Table 1 gives a possible range of density, temperature and pressure for the case $\kappa R = 9$. Mean density $\bar{\rho} = 1.3 \text{ kg m}^{-3}$ and surface temperature $T_s = 4,000 \text{ K}$ served as reference for the calculation. The surface layer was treated as an ideal gas with atomic weight 14.2, and the central plasma was taken as fully ionised air.

Table 1 Range of parameters derived from hydrostatic equilibrium in an isobaric ball lightning model with $\kappa R = 9$

Parameter	Centre	Surface
Density	$\rho_C = 3.8 \times 10^{-5} \text{ kg m}^{-3}$	$\rho_s = 7.8 \text{ kg m}^{-3}$
Temperature	$T_C = 1.0 \times 10^8 \text{ K}$	$T_s = 4.0 \times 10^3 \text{ K}$
Pressure	$p = 181 \text{ atm}$	$p = 181 \text{ atm}$

The hydrostatic temperature profile of Table 1 is bound to be smoothed by energy transfer from the hot central region to the cooler surface layers. The mode of charge transfer and its effect on the equilibrium conditions in the ball lightning model are charged vortices in a turbulent plasma with: (1) electrical properties of a superconductor; (2) binding energy from density stratification; (3) energy storage in the pressure field.

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Electron-optical observations of ordered FeNi in the Estherville meteorite

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Scott and Clarke¹, using optical techniques, have recently reported the presence of ordered FeNi (taenite) with the tetragonal $L1_0$ structure (AuCu) in over 40 meteorites. The ordered phase occurs in 10–60 μm diameter regions and in 1–5 μm wide rims of clear taenite containing 48–57% Ni (refs 1, 2). In contrast with disordered γ -Fe-Ni, the ordered taenite is optically anisotropic. The X-ray diffraction pattern and Mössbauer spectrum of ordered FeNi^{3–5} are also similar to that of tetragonal FeNi synthesised using high flux neutron irradiation at 300 °C of a disordered FeNi alloy in the presence of a magnetic field.

Although Mössbauer and X-ray diffraction studies^{6,7} have been used to detect superstructure in bulk samples, these techniques cannot spatially locate the ordered phase within the microstructure. The present study uses various electron-optical techniques, including transmission electron microscopy (TEM), scanning transmission electron microscopy (STEM) and electron probe microanalysis (EPMA) to characterise and to resolve the ordered FeNi phase spatially. In addition, the first TEM evidence (both electron diffraction patterns and centred dark field (CDF) images) for superstructure in clear taenite, is presented.

A thin section of the Estherville meteorite containing a large area of continuous anisotropic taenite ($240 \times 500 \mu\text{m}$) was obtained from the Museum of Natural History, Smithsonian Institution, Washington, DC. The thin section also contains other areas that show optically anisotropic taenite in the form of $5 \mu\text{m}$ wide borders or as small patches that are often associated with schreibersite. Under crossed polars the large taenite field (Fig. 1) shows an intricate network of sub-domains and an irregular granular structure which is characteristic of anisotropic clear taenite^{1,2}. The EPMA trace (Fig. 1 inset) shows an approximately uniform composition across the taenite field. The average Ni content of the taenite is $50 \pm 2\%$ and this value is within the range 48–57% obtained for clear taenite^{1,2}.

Subsequent to the optical and EPMA studies the ($240 \times 500 \mu\text{m}$) clear taenite was extracted from the thin section for TEM and STEM investigations. Adopting a procedure outlined by Nord and James⁸, the clear taenite area was thinned to electron transparency by argon ion bombardment. The electron transparent thin foil was examined in a Philips EM300 electron microscope operating at 100 kV. Using a STEM attachment and X-ray energy dispersive spectrometry (EDS) localised chemical compositions were measured with an X-ray spatial resolution $\leq 50 \text{ nm}$.

TEM examination of the thin foil reveals areas that show characteristically different ion etching behaviour. Several 1–3 μm diameter preferentially thinned regions are observed in a

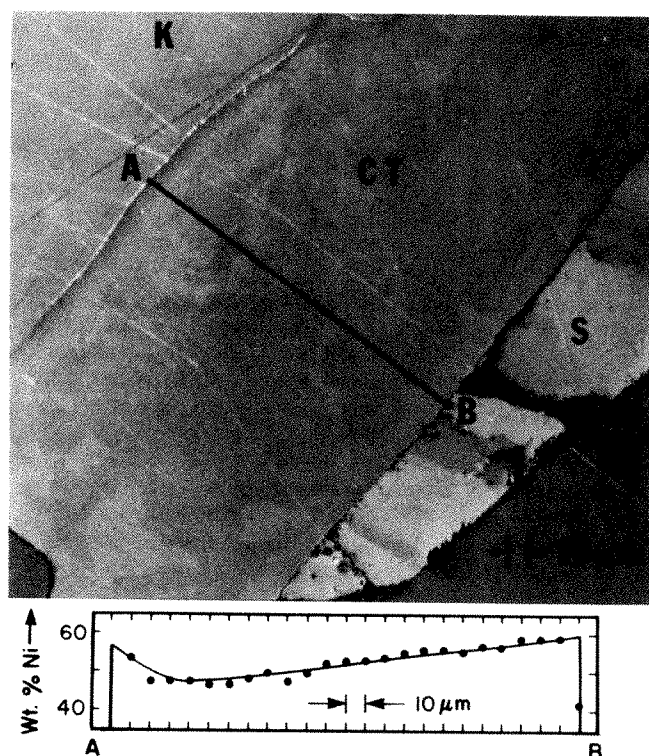


Fig. 1 Crossed polar reflected light photomicrograph of clear taenite (CT) surrounded by sulphide (S) and kamacite (K) in the Estherville meteorite. Notice the optical anisotropy (indicated by regions of contrast) in clear taenite. Electron probe Ni concentration profile taken across clear taenite is shown in the inset.

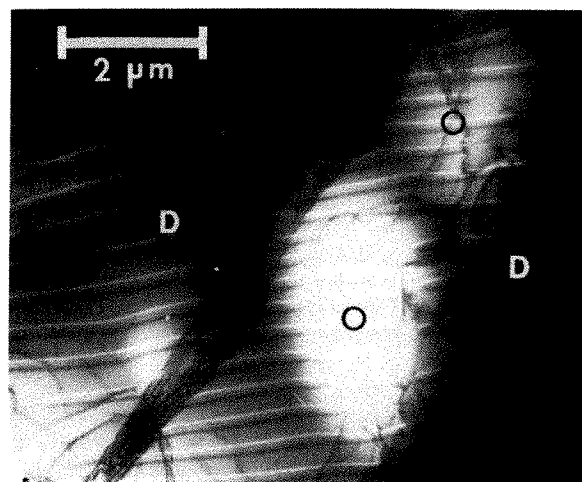


Fig. 2 Low magnification transmission electron micrograph of the ion thinned foil of clear taenite shown in Fig. 1. Notice preferentially thinned ordered regions (O) surrounded by disordered, or different ordered variant matrix (D). Lamellar features crossing both regions are magnetic domain walls imaged in slightly defocused conditions.

relatively thick matrix (Fig. 2). As the clear taenite is chemically homogeneous we suggest that the preferential ion etching of certain areas within clear taenite is structural rather than chemical in origin. The parallel black and white lines in Fig. 2 are characteristic of magnetic domain walls imaged in the TEM, and indicate the ferromagnetic nature of both the preferentially thinned and thicker areas.

Electron diffraction from several 20 μm selected areas indicates that the thin foil is a single crystal of taenite. In bright field images various structural defects are observed. These defects have the characteristics of twin boundaries, Neumann bands and a dense dislocation substructure. They are concentrated in the matrix regions and probably formed as a result of shock.

Electron diffraction patterns from all preferentially thinned regions show superlattice reflections in the fundamental FeNi

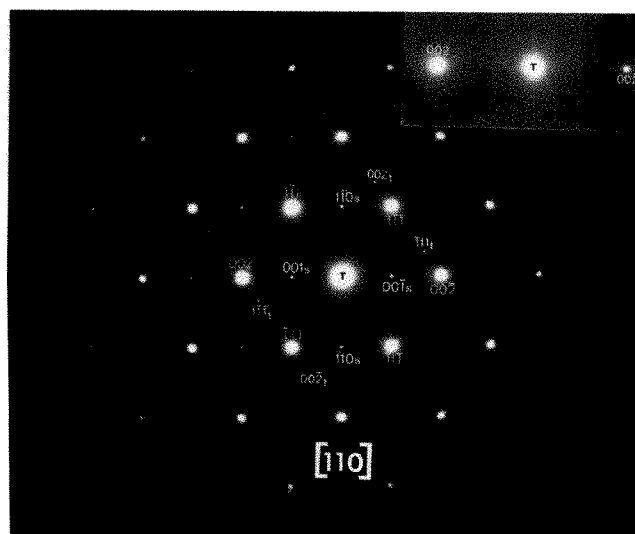


Fig. 3 Electron diffraction pattern taken from a preferentially thinned region in clear taenite (Fig. 2). The beam direction is $[110]$. The spot marked T is the transmitted spot, those marked S are the superlattice spots. The pattern also shows twin spots marked t which identify the twinning in the matrix as the conventional f.c.c. variety with the twin plane $K_1 = (111)$. Note the absence of superlattice spots in the inset pattern taken from the adjacent matrix.

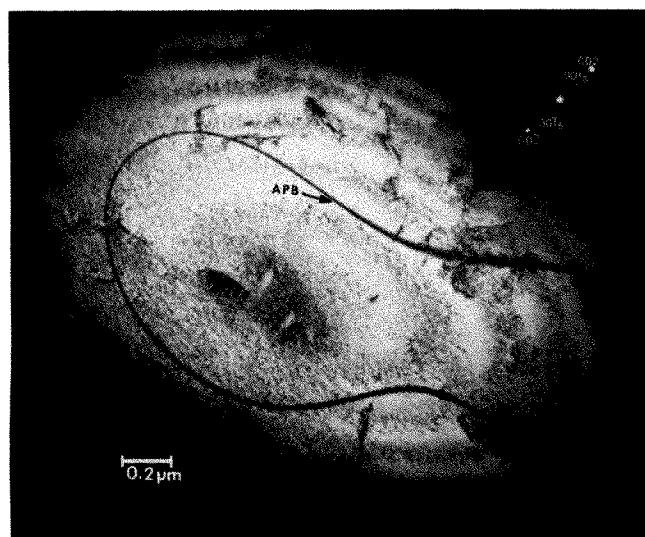


Fig. 4 Antiphase domain boundary (APB) in the ordered region of clear taenite shown in Fig. 2. Centred dark field image with (001) superlattice reflection. The accelerating voltage is 100 kV. The corresponding electron diffraction pattern is shown in the inset.

(f.c.c.) reciprocal lattice. The superlattice reflections arise from long-range ordering in these regions and indices can be assigned to these reflections on the basis of the LL_0 tetragonal structure. Figure 3 is a symmetric (110) zone axis pattern from an ordered region clearly showing (001) and (110) superlattice reflections. Absence of ordering in the adjacent matrix regions is indicated by disappearance of the superlattice spots in the diffraction pattern (Fig. 3, inset). (The electron diffraction pattern also shows faint twin spots which obey the conventional f.c.c. twinning law with (111) as the twinning plane. Twins were present across both ordered and disordered regions.) As a further confirmation of ordering, CDF images using superlattice spots were used to show the presence of antiphase domain boundaries (APB) within the ordered regions. Figure 4 shows a CDF image obtained with the (001) superlattice reflection. An APB shows up as a dark fringe running across the foil surface in the form of an open loop⁹. We propose, therefore, that clear taenite in the Estherville meteorite contains regions of ordered FeNi phase in a disordered γ matrix. The adjacent areas showing no superlattice spots are either disordered or alternatively (E. R. D. Scott, personal communication) are ordered LL_0 domains in which the {001} ordered planes are in another of the three possible, mutually perpendicular, orientations that could exist and hence are not correctly oriented for diffraction. It was not possible to distinguish between these two explanations since the specimen would have to be tilted to either a (100) or another (011) orientation to observe a different set of superlattice spots. In the natural orientation of this specimen this was not possible. STEM X-ray microanalysis from the ordered and adjacent regions indicates that both have the composition $52 \pm 2\%$ Ni.

Evidence for the presence of coexisting ordered and disordered taenites in other meteorites has also been provided by Mössbauer and X-ray diffraction studies of Albertsen *et al.*⁴. However, their study shows 28% Ni content for the two taenites in which the disordered taenite is paramagnetic. This composition is clearly below that range obtained for clear taenite^{1,2}. More recently, electron microscopy of cloudy taenite in Santa Catharina meteorite by Jago¹¹ revealed two taenites associated with kamacite in a structure which apparently is a precursor to cloudy taenite. Jago¹¹ further reports that one of the taenites became ordered on heating to 400 °C.

We have presented direct TEM evidence for ordering of FeNi in clear taenite. No evidence was obtained for any crystallographic discontinuity between ordered regions and their surrounds. Therefore description of the optical anisotropy in terms of 'ordered grains' (refs 1, 2) is considered inaccurate. There is

also a clear distinction between TEM and Mössbauer results, since in the latter there is no evidence for the presence of both ferromagnetic taenite and ordered FeNi in the same taenite field⁴. This inconsistency must be explored further. The ordered phase may have formed when the parent meteorite body cooled below the ordering temperature of the superstructure ($T_c = 320$ °C) as suggested by Danon *et al.*¹⁰.

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Determination of crystal structure of metastable anthracene by a novel method

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A combination of transmission electron microscopy (TEM) and 'atom-atom' computational procedures^{1,2} developed for molecular crystals has led to the discovery and the determination of the crystal structure and lattice dynamics of a metastable, triclinic phase (hereafter designated, II) of anthracene. The new form, which is only 2 kJ mol⁻¹ less stable than the monoclinic, thermodynamically stable parent phase (I), may be generated by the application of compressive force or shear stress approximately perpendicular to the basal plane of I. In essence, the size and shape of the unit cell into which molecules of anthracene are packed is determined from the electron diffraction patterns of the new phase. From the symmetry and space group, determined by TEM, assuming that the individual molecules are as undistorted in II as in I, a 'trial structure' of the 'new' phase may be computed by minimising the total energy as a function of the molecular packing characteristics, knowing the empirically determined³ atom-atom potentials between all pairs of non-bonded atoms. The structure is refined by varying the molecular coordinates, as described below, so as to arrive at a set of lattice vibrations^{4,5} all of which are real in the entire Brillouin zone. The enhanced photo reactivity of phase II compared with I is explicable in terms of the resulting crystal structure.

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We recently reported⁶ the facile production of a metastable phase of anthracene which was discovered from analysis of the electron diffraction patterns of anthracene (I) that had suffered stress at room temperature. Phase II is produced topotactically⁷ inside I such that $(001)_{II} \parallel (001)_I$, $(100)_{II} \parallel (101)_I$ and $(010)_{II} \parallel (011)_I$. Moreover, the conversion of I leads to a coexistent bi-phasic matrix of I and II; irradiation of this matrix with UV light rapidly results in the solid state formation of dipara-anthracene (by formation of $C_9-C'_9$ and $C_{10}-C'_{10}$ single bonds at the 'meso' positions of neighbouring molecules).

From the topotactic relationships quoted above, a set of unit-cell dimensions for II may be derived: $a = 8.56$, $b = 6.04$ and $c = 11.22$ Å, $\alpha = 122.6$, $\beta = 101.2$ and $\gamma = 90.0^\circ$ (in terms of the reported crystal structure⁸ of I). But when the standard energy minimisation procedure using appropriate³ 'non-bonded' potentials of the form: $U_{ij} = A/r_{ij}^6 + B \exp(-Cr_{ij})$, was used the resulting structure, although it had a lattice energy only marginally less than that of I, possessed imaginary frequencies for a few lattice vibrations, thereby indicating that this trial structure was untenable. We therefore explored the energetics and dynamics of a range of structures based on slight distortions of this trial structure. We set an upper-limit of $\pm 5\%$ for the degree of mismatch between the cell dimensions of I and II—a figure which is reasonable in view of the absence of observed electron-microscope strain contrast at interfaces of II and I. From the many sets of unit-cell dimensions for II that may be generated on this basis there emerged, after packing analysis (energy minimisation) and lattice dynamical calculations, either: (1) dynamically unstable structure(s); or (2) the stable monoclinic $P2_1/a$ phase (I) or (3) a triclinic structure (space group $P\bar{1}$, $Z = 2$ and cell constants $a = 8.342$, $b = 5.892$, $c = 11.282$ Å, $\alpha = 123.34$, $\beta = 96.70$ and $\gamma = 85.91^\circ$) with no imaginary lattice frequencies (the minimum energy structure quoted in ref. 6 has subsequently been found to be dynamically unstable). Full details of the structure are given elsewhere⁹, but the salient difference is the intermolecular disposition of the two molecules situated at $(1, 0, 0)$ and $(\frac{1}{2}, \frac{1}{2}, 0)$ as shown in Fig. 1. This new phase II, which does not seem to exist in the bulk phase, readily reverts to phase I in normal conditions of temperature and pressure: at low temperatures its lifetime may be prolonged. As the temporary stabilisation of II could arise from the presence of the topotactic interfaces with I, we have also looked at their energetics and structure using the non-bonded potentials and the energy minimisation procedure.

Using a computational approach in which layers of I and II on either side of an imaginary interfacial plane are in coherent contact (zero mismatch), with lateral mismatches progressively increasing with distance away from the interface, it is possible to estimate the 'excess energy' at the three principal boundary planes that give rise to the topotaxy. Again a pairwise evaluation of non-bonded interactions is undertaken. The 'excess energies' at all three topotactic interfaces are small (roughly a tenth of the surface energy of the respective plane), unlike the typical excess energies at high- and low-angle boundaries in metals (which are, respectively, about a third and a sixth of the corresponding surface energies). Besides the favourable energetics at the interface, we also find that the intermolecular contacts and molecular conformation across the interfaces are analogous to those found within the bulk structures of I or II. These results are in line with our observations in the electron microscope where we have found no direct evidence for mismatch dislocations, strains or clear boundaries between the two phases.

Careful control over the structural and chemical purity of the anthracene crystals and subsequent photo-reaction studies (in the TEM), reveal unmistakably the positive influence of the new phase on dimer formation. Enhanced photoreactivity is exhibited at the compressed regions of the crystal.

Significantly, compared with the situation that prevails for phase I, some of the intermolecular dispositions are different in phase II and at interfaces of I and II. Of particular relevance is the shortening of both the $C_9-C'_9$ and $C_{10}-C'_{10}$ distances between the adjacent molecules $(1, 0, 0)$ and $(\frac{1}{2}, \frac{1}{2}, 0)$ on proceeding from I

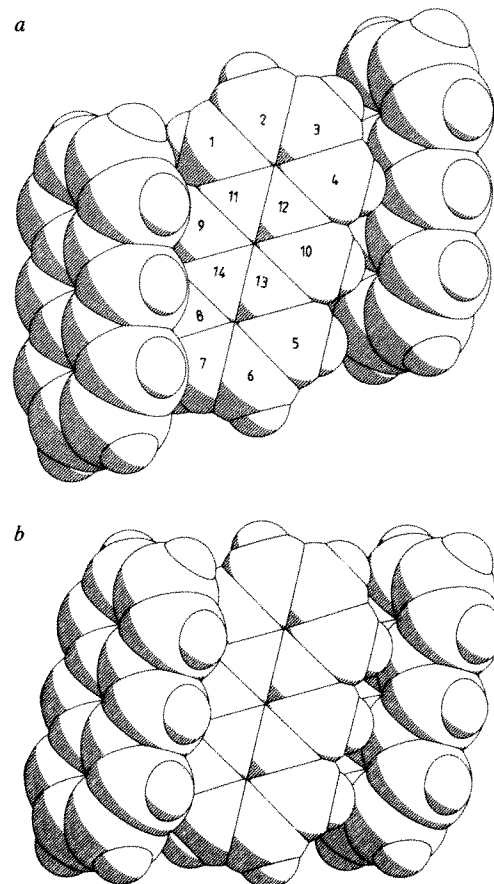


Fig. 1 Illustration of mutual orientation of nearest molecules in phases I (a) and II (b) or crystalline anthracene. Only three molecules in the $(\bar{1}10)$ plane are shown.

to II. The green fluorescence observed in the compressed specimens may also be attributed to the slightly increased overlap between these molecules in II. Also of relevance is the relative ease with which a molecule of anthracene at $(\frac{1}{2}, \frac{1}{2}, 0)$ in phase II may be rotated (with limited hindrance from surrounding molecules) about its long axis, thereby bringing it face-to-face and overlapped with its adjacent molecule at $(1, 0, 0)$. Both these factors will render II more susceptible than I to photodimerisation, in line with observation.

Thus, the present method can be used to elucidate structural information from basic TEM data, starting with a static calculation procedure (lattice-energy minimization) followed by a further refinement using a dynamic treatment (phonon dispersion). Notwithstanding the limits imposed by the approximations inherent in the calculation methods used, it is anticipated that this approach to crystal structure determination should be widely applicable to other organic crystals, especially where problems of small crystal size and the occurrence of polymorphic intergrowths are encountered.

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Polar ice evidence that atmospheric CO₂ 20,000yr BP was 50% of present

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Analysis of the air enclosed in polar ice (~0.1 ml per g of ice¹) provides one of the most promising ways of discovering atmospheric composition over the past 100,000 yr. Until now, because of an apparent but not well understood enrichment of CO₂ in the trapped air, all attempts to reconstitute the ancient atmospheric CO₂ content from polar ice have failed²⁻⁶. We have obtained CO₂ contents that can reasonably be considered representative of present or past atmospheric contents using a new method of air extraction. The results reported here, based on the CO₂ analysis of two deep Antarctic cores including the last climatic interchange, strongly suggest that during the coldest part of the last Ice Age (20,000–15,000 yr ago) the atmospheric CO₂ content was half (0.016%) that of today's level (0.033%).

Carbon dioxide extracted in a vacuum from melted ice samples can originate from: (1) air contained in bubbles within the ice; (2) gas which may be trapped or adsorbed by the ice lattice; or (3) the decomposition of dissolved carbonates or carbonate particles which are present naturally or artificially (through contamination) in the solid.

Raynaud and Delmas have previously determined the CO₂ content of ice cores from several polar sites (Greenland and Antarctica) and of various ages (Holocene and Wisconsin periods)⁵. All these determinations showed a large CO₂ enrichment with respect to the present-day composition. The general mean was 0.35% at Camp Century (Greenland) and 0.13% at Byrd Station (Antarctica). We concluded that the gas-solid interaction in the firn is probably responsible for this phenomenon. However, we pointed out that a modification of the initial CO₂ content during storage of the ice core could not be excluded. More recent results⁷ concerning the detailed analysis of an ice core representing ~2 yr precipitation at Camp Century also show a high enrichment of CO₂ (content, 0.1%) in the extracted air.

Table 1 CO₂ in Antarctic ice (% in the total gas extracted)

Site	Depth (m)	No decontamination	% of CO ₂ Decontaminated	Decontamination method
D10	113	0.61 (1)	0.034 (6)	b
66°40' S, 140°01' E	148		0.036 (4)	b
270 m, -19 °C, 0.123	226		0.032 (5)	b+d
	226		0.021 (2)	b
	248		0.038 (1)	b
Dome Summit	100	0.13 (32)	0.037 (1)	b
66°17' S, 110°32' E				
1,390 m, -22 °C, 0.115				
Dome C (DC)	114		0.042 (2)	a
74°39' S, 124°10' E	137		0.037 (3)	b+c+e
3,240 m, -53 °C, 0.0845	249	0.32 (1)		
	300	0.28 (1)		
	330		0.054 (1)	a
	473	0.22 (1)	0.074 (1)	b
	473		0.050 (2)	a
	589	0.105 (1)	0.051 (1)	a
	589		0.048 (1)	b
	670		0.067 (1)	a

Results without, and after decontamination of the ice (see text). For each site elevation (m), mean annual temperature (°C) and total gas content in the depth range 100–150 m are given (ml per g of ice) (from ref. 8). Number of determinations are given in parentheses. Decontamination methods: a, water, 20 °C; b, ethanol, -15 °C; c, acid bath, 20 °C; d, ultrasonic acid bath, 20 °C; e, acid bath -12 °C.

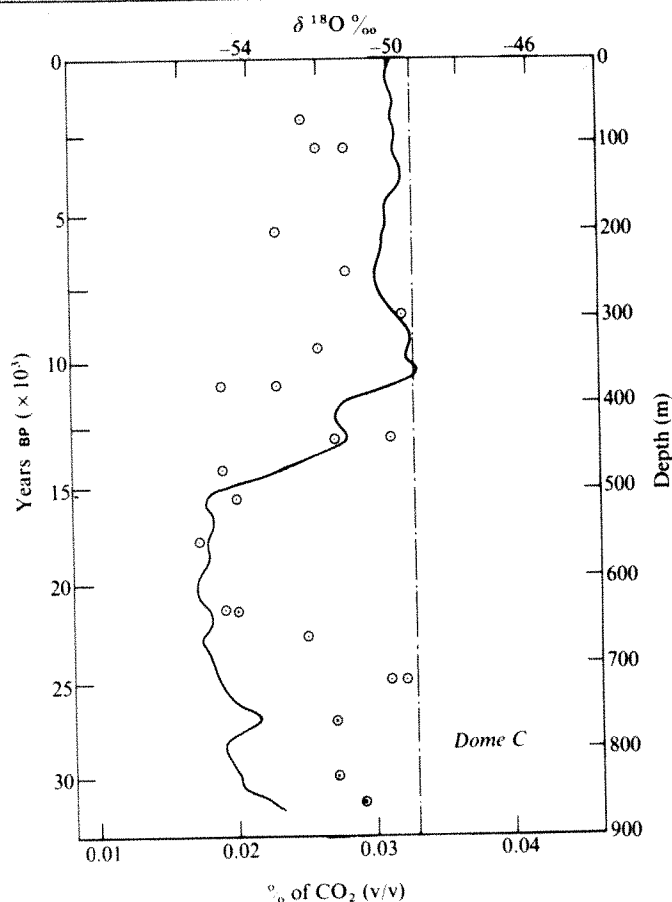


Fig. 1 % of CO₂ (v/v) in the air of the bubbles from the Dome C ice core against depth (in metres of ice equivalent). Isotopic values ($\delta^{18}\text{O}$ ‰ continuous line) and dating are as published previously¹⁰. Note the low CO₂ contents between 22,000 and 15,000 yr BP. The present day atmospheric CO₂ content (0.033%) is indicated by a dashed line.

Classical gas extraction procedures and CO₂ content determination have been described previously^{5,8}. The ice core is cut in a cold room with an electrical band saw. All faces of the sample (a cube of 30–50 g) are 'new' to minimise contamination. The sample is placed in a glass vessel which is then mounted in a vacuum line. After melting of ice, CO₂ content is determined in the extracted gas chromatographically. We have investigated first the contribution of carbonates (actually bicarbonates HCO₃⁻) which could be responsible for the CO₂ enrichment. On the basis of a 0.03% CO₂ content in the gas extracted, 1,000 g of ice contains ~30 µl of CO₂ whereas 1 µEquiv. of HCO₃⁻ releases through decomposition 22.4 µl of CO₂. This comparison shows that the total CO₂ measurements are very sensitive to the possible presence of small amounts of carbonates in the lattice. For 32 samples of Dome Summit (66°17'S, 110°32'E) for which the 'classical' gas extraction method gave a mean value of 0.13% of CO₂ ($\sigma = 0.07$), we determined, by a sensitive (± 0.2 µEquiv. l⁻¹) titrimetric method, a surprisingly high mean bicarbonate content of 7 µEquiv. l⁻¹. As estimation of the natural carbonate content leads to much smaller values, contamination of the samples could be responsible for this high carbonation. (The molar ratio HCO₃⁻/Na is 0.005 in sea salt, assumed to be the major impurity (some µEquiv. l⁻¹) in snow at this coastal site.) By rinsing copiously the surface of the solid sample with double permuted water or ethylalcohol we considerably reduced the carbonation.

New determinations of CO₂ in decontaminated polar ice were made as for the carbonate measurements. The results (Table 1) show clearly that the gas extracted from the ice has a CO₂ content which is not significantly different from the present-day air composition. These results contrast strongly with the former reported values. Our experiments explain *a posteriori* the poor reproductibility of the previous CO₂ measurements, the

difficulty of extracting the total CO_2 from meltwater and the high pH values of the meltwater sometimes observed⁷.

To eliminate more completely the contamination problems linked with the ice lattice, we developed a dry extraction method. The solid ice sample (~40 g) was pulverised under vacuum at -40°C in a closed stainless steel container with the aid of two metallic balls for 30 s. About 75% of the trapped gases are released. They were then transferred to the gas chromatograph, as for a wet extraction. We checked that our CO_2 concentration measurements were independent of grinding time, of gas extraction rate, as well as of the state of the sample (contaminated or fractured).

Two deep Antarctic ice cores have been analysed by this method (Dome C and D10). The results (Table 2) are plotted against depth and age in Figs 1 (Dome C) and 2 (D10). The average CO_2 concentration was 0.025% at both locations, that is, much less than published to date and still slightly lower than values obtained using the classical extraction method after decontamination (Table 1) particularly for the deepest fractured

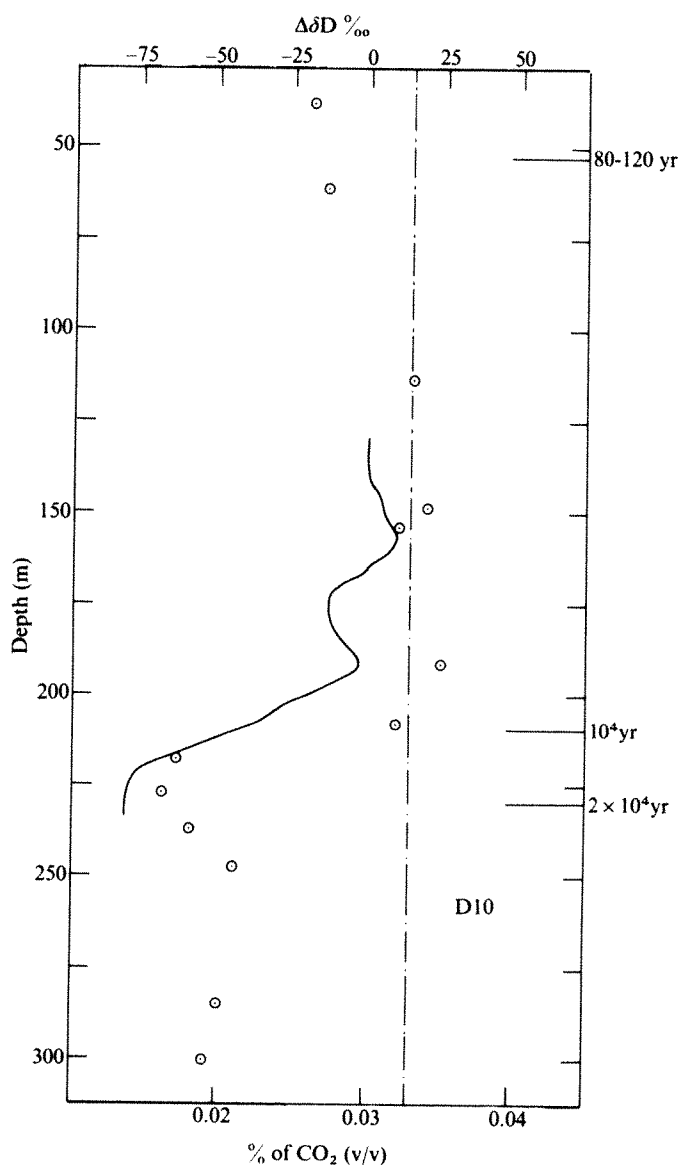


Fig. 2 % of CO_2 (v/v) in the air of the bubbles from the D10 ice core against real depth (in metres). The interpretation of the isotopic profile ($\delta\text{D}\text{‰}$, continuous line) is complicated in this coastal site by glacier flow problems. Climatic fluctuations are given here in terms of variations of $\delta\text{D}\text{‰}$ ($\Delta\delta\text{D}\text{‰}$). They have been reconstituted by Raynaud *et al.*⁹ and apparently include the last climatic interchange. Low CO_2 contents are observed during the coldest period (215–235 m). The present-day atmospheric CO_2 content (0.033%) is indicated by a dashed line.

Table 2 CO_2 content of ice air bubbles at DC and D10 (Antarctica)

Depth (m)	DC % CO_2	$\pm\sigma$ ($\times 10^3$)	Depth (m)	D10 % CO_2	$\pm\sigma$ ($\times 10^3$)
103	0.025	2.0	37	0.026	2.0
137	0.028	3.4	61	0.027	2.3
	0.026	3.2	113	0.033	2.7
232	0.023	2.0	148	0.034	2.7
277	0.028	2.8	154	0.032	2.5
330	0.032	2.7	191	0.035	2.8
366	0.026	3.0	208	0.032	3.3
410	0.023	2.6	218	0.017	1.6
	0.019	2.6	227	0.016	2.3
472	0.031	2.7	237	0.018	2.1
	0.027	2.8	247	0.021	2.5
507	0.019	2.6	285	0.020	2.3
541	0.020	2.8	301	0.019	2.3
589	0.017	2.6			
670	0.020	2.8	Overall mean value	0.025	0.72
	0.019	2.8			
701	0.025	3.6			
752	0.031	2.3			
	0.032	3.9			
800	0.027	4.0			
864	0.027	3.9			
893	0.029	4.1			
Overall mean value	0.025	0.46			

The standard deviation (s.d.) is given for 95% confidence limits.

(and hence possibly contaminated) ice samples. In some cases (113 and 148 m, D10), nearly identical results were obtained (0.033–0.036%) by using either analytical method—a good indication that CO_2 is located essentially in the bubbles. On the other hand, the 'dry' extraction method seems to be the only one convenient for CO_2 analysis of fractured ice cores (even if they have lost important volumes of gases—as is the case for the two cores we studied from a depth below 200 m).

The most interesting feature in Fig. 1 is the marked CO_2 depletion (0.016–0.020%) during the coldest part of the glacial age (15,000–20,000 yr BP). The same is observed at D10, although the dates of the ice layers are still being questioned at this location. The temperature increase (estimated at DC to be $\sim 7^\circ\text{C}$ (ref. 10)) which followed the end of this period is accompanied by a similar trend for CO_2 . However, our results are not accurate enough to conclude whether the increase in atmospheric CO_2 content preceded or proceeded the climatic change. A time lag could exist between the age of the ice and the age of the entrapped gases because the interstitial air of the firn is probably in contact with the free atmosphere for a long time (up to 2,000 yr at Dome C). On the other hand the interstitial air could be also contaminated by older air expelled from deeper firn layers by the slow compaction process. The net result of such effects could be a smoothing of the variations of the initial atmospheric CO_2 content. During the Holocene, the mean values (0.031% at D10, 0.027% at DC) are relatively consistent and essentially in agreement with the estimated pre-industrial values (0.027–0.029%)^{11,12}. The variations of the CO_2 content during this period, particularly at Dome C (six depth levels studies), can be explained by the reported analytical uncertainties (Table 1). Nevertheless, at both locations, the highest CO_2 contents of the Holocene period are obtained around the climatic optimum which immediately follows the climatic interchange¹⁰. The results concerning the oldest samples (ages > 20,000 yr) are difficult to interpret due to dating uncertainties, especially at D10.

Finally although physicochemical processes within the firn (such as adsorption) could also affect the composition of the air trapped in the polar ice^{5,13}, these new results show that the importance of the CO_2 enrichment found in the polar ice gases has been largely overestimated and that the ancient variations of the atmospheric CO_2 content can most probably be reconstructed from deep ice cores.

Berner *et al.*¹⁴ have recently analysed two deep ice cores (Camp Century, Greenland and Byrd, Antarctica) for CO₂ content. Their experimental procedure for extracting CO₂ from the ice was similar to our 'classical gas extraction method'. They consider two different CO₂ fractions (the gaseous CO₂ and the CO₂ in the ice lattice), and their results strongly suggest that the atmospheric CO₂ concentration during the last glaciation could have been a factor of 1.5 lower than today, which is in good agreement with our own results.

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Recent climatic trends and local glacier margin fluctuations in West Greenland

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Recent climatic trends in the Arctic have been characterised by a general cooling between the mid-1950s and the late-1960s, followed by a return to warmer conditions in the early 1970s (refs 1, 2). Throughout the Canadian Arctic Archipelago and at Thule in north-west Greenland a marked decrease in summer temperature occurred after 1963, and winter precipitation increased^{3,4}. These changes were accompanied by a lowering of the average July freezing level height by as much as 500 m (ref. 5), decreased glacier mass loss⁴ and increased glaciation³. Here I report similar climatic trends in West Greenland and demonstrate different glacier responses, in particular an advance of cirque and small valley glaciers since about 1968, contrasting with a simultaneous retreat of larger valley and icefield outlet glaciers.

Mean annual and seasonal temperature and precipitation trends for Godthåb (64°10'N, 51°33'W) since 1920 are presented in Figs 1 and 2, based on data provided by the Meteorologisk Institut in Copenhagen. Summer is defined as the months May to September; winter as the months October to April. These groupings approximately encompass the glacier ablation and accumulation seasons, respectively. Spring and autumn are broadly equated with the months May and September, respectively. A major feature of the temperature graphs is the marked cooling in all seasons from about 1966 to 1971 followed by a trend towards warmer conditions after 1971, except in winter. During 1971, 5-yr mean summer temperatures reached their lowest level since the start of continuous records in 1875. Winter and summer precipitation increased sharply in the 1950s and

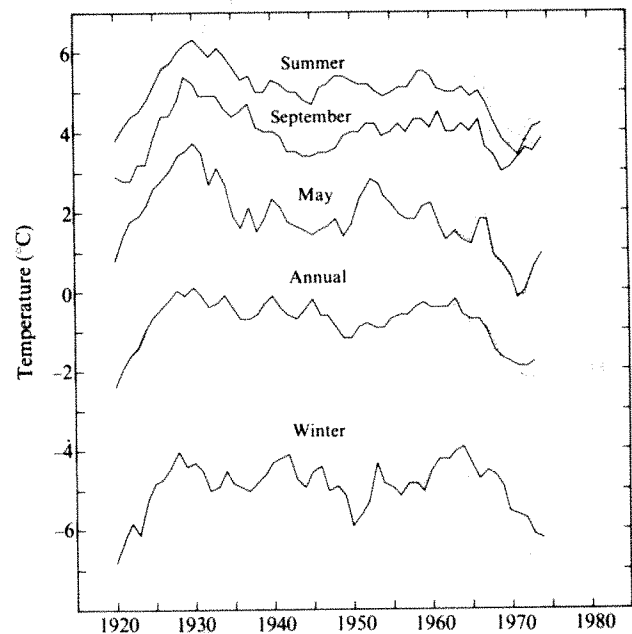


Fig. 1 Five-year running means of temperature at Godthåb (solid lines) and Sukkertoppen (dotted lines). Data are plotted for the middle year of each five year period. Winter data are plotted for the second year of each winter.

1960s, and the highest 5-yr means since the start of continuous records in 1920 were in 1966 and 1963, respectively. Thereafter, precipitation totals fell, but remained above those for the years 1920 to 1960. Climatic data for Sukkertoppen, 145 km north of Godthåb and the nearest town to the glaciers investigated, are only available from 1960 onwards, but the trends from this date are similar to those for Godthåb (Figs 1 and 2). The origin of the above trends has been related to the development and persistence of a ridge of pressure anomaly over Greenland in the 1950s and 1960s and its sudden collapse after the winter of 1970–71². Manifestations of the climatic deterioration in the form of increased ice-cover of the sea and biological changes have already been reported^{1,2}. I now describe some glaciological effects.

The area at the head of Íkamiut kangerdluarssuat (Fig. 3), 45 km north of Sukkertoppen, is one of spectacular alpine-type glaciation: many cirque and small valley glaciers up to 6 km long

Table 1 Glacier locations, sizes and icefront changes

Glacier	Location		Length (km)	Overall change in glacier front position between 19th century ice maximum extent and 1968–69		Overall change in glacier front position between 1968–69 and 1978
	lat	long		(m)	(m)	
1	65°49'36" N	52°39'47" W	3.5	–1,260	+82	
2	65°45'20" N	52°40' 7" W	2.3	–480	+50	
3	65°46'41" N	52°46'57" W	1.5	–540	+46	
4	65°47' 0" N	52°50'52" W	2.2	–730	+20	
5	65°46'21" N	52°34' 5" W	2.5	–690	+117	
6	65°48' 6" N	52°34'44" W	6.0	–1,200	–34	
7	65°41'42" N	52°37'24" W	2.0	–800	+158	
8	65°42'26" N	52°36' 6" W	10.0	–1,500	–190	
9	65°44' 8" N	52°35'56" W	7.5	–1,180	–55	

Glaciers 1 to 7 are cirque or valley glaciers; glaciers 8 and 9 icefield outlet glaciers.

are set among mountains rising steeply from sea level to a maximum altitude of 1,755 m, and several outlet glaciers drain an icefield approximately 40 km by 15 km in size at an altitude of about 1,200 m above sea level and existing independently of the Inland Ice. Before about 1968 the glaciers of the Sukkertoppen area had been progressively retreating, although interrupted by several short halts, from the advanced positions of their historical maximum extent in the mid to late nineteenth century⁶. This pattern has been confirmed for seven cirque and valley glaciers and two outlet glaciers investigated during the summer of 1978⁷. The positions of these glaciers in relation to prominent points in their forelands were compared with those on vertical aerial photographs taken in 1968 in some cases and 1969 in others by the Geodætisk Institut, Copenhagen. The two outlet glaciers and the largest valley glacier have continued to recede overall between 1968–69 and 1978 by between 34 and 190 m (Table 1). However, in two of the forelands (6 and 9 in Table 1) the presence of end moraine ridges between the 1968–69 and 1978 ice margin positions indicates temporary reactivation at some stage during this period. In the case of glacier 9 the ridge is 3.5 m high, and the volume of debris clearly represents several years accumulation when compared with that seen melting out from the glacier in 1978. As two recessional ridges superimposed on its proximal slopes are probably push moraines formed during the winters of 1977–78 and 1976–77, I infer that the main ridge was formed between about 1971 and 1975. It is unlikely to have been formed much earlier as it lies inside the 1969 ice margin position. The glacier has since receded by up to 10 m from the main ridge crest. Neither plants nor lichen were observed in the immediate proglacial areas of the three receding glaciers.

In contrast, six of the cirque and valley glaciers have advanced overall from their 1968 or 1969 positions by distances of between 20 and 158 m (Table 1). When visited, they were generally in close contact with fresh moraine and boulder ridges at their margins, and the advance was probably still in progress. In two cases slight recession of 1 to 2 m from the ridges may

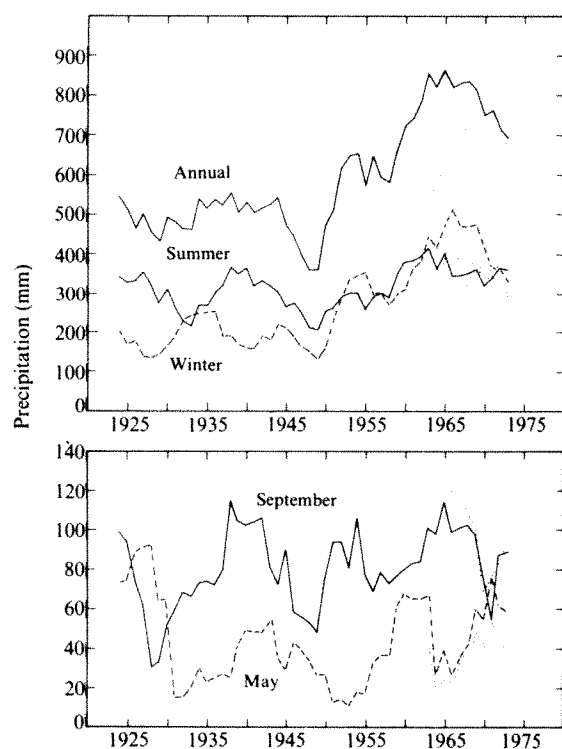


Fig. 2 Five-year running means of precipitation at Godthåb (annual, summer and September, solid lines; winter and May, dashed lines) and Sukkertoppen (dotted lines). Data are plotted for the middle year of each 5-yr period. Winter data are plotted for the second year of each winter.

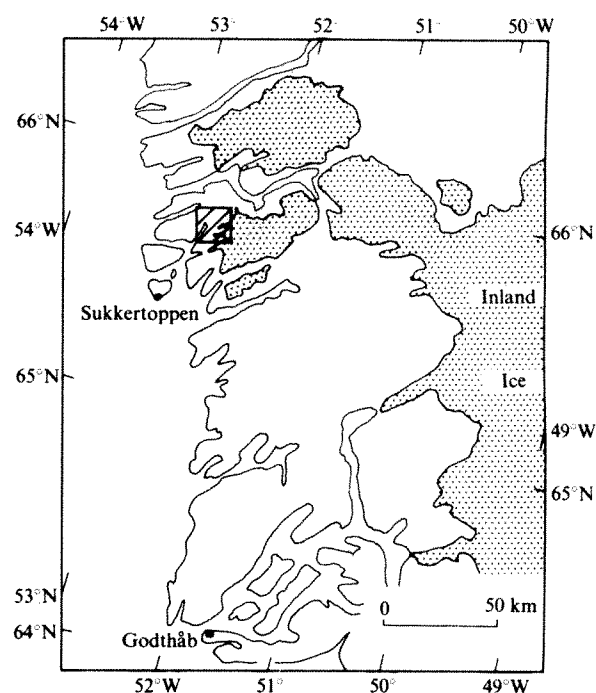


Fig. 3 Location map. The glaciers investigated occur within the shaded square north of Sukkertoppen. Major glaciers only are shown by stippling.

reflect negative summer balances as observations were not made at the start of the ablation season. Plants and patches of moss were often incorporated or disturbed at the distal edges of the marginal debris ridges. Lichen of *Rhizocarpon* species with thalli up to 9 mm in diameter (equivalent to an estimated age of 22 yr) occurred within 15 m of some of the glacier fronts.

The recent frontal changes of the cirque and small valley glaciers are sufficiently similar in direction and timing to argue that they relate to the changing climatic conditions rather than internal instabilities or surges. I therefore comment on possible relationships between the climatic trends and the general response of the glaciers. Theoretically the glaciers will respond relatively rapidly to changes in temperature or radiation conditions because of the immediate effects on ablation in the sensitive snout areas, but there will tend to be a lag in their response to changing precipitation conditions while the effects are propagated down-glacier from the accumulation areas.

The advance of the cirque and small valley glaciers since about 1968 coincides in its first part with a period of decreased summer temperatures and a trend towards colder, wetter springs and colder, drier autumns, and can be seen as a direct response to this climatic deterioration. However, despite a reversal in all these trends after 1971, the glaciers have continued to advance at least until 1978. Therefore, the observed pattern of glacier margin fluctuations matches only in part the recent temperature trends. The continued advance of the glaciers probably reflects a lag in their response to the increased precipitation during the late 1950s and 1960s. The magnitude of this lag is at least 9 yr judging from the fact that precipitation began to decrease sharply after 1969.

The temporary reactivation interrupting the continued recession of one of the outlet glaciers and the largest valley glacier can be explained as a direct response to the climatic cooling, particularly in summer seasons during the late 1960s and early 1970s. However, these glaciers do not seem to have responded significantly to the increased precipitation in the 1950s and 1960s. This could reflect either a lag or a damping of any effects due to the size of the glaciers. The former is more probable because of the magnitude and duration of the precipitation increase. The continued recession of the two outlet

and the largest valley glaciers at present can be explained partly as a direct response to the recent climatic warming and partly as a lag response to the relatively low precipitation during the 1940s and early 1950s. If this is the case, then a minimum lag of 20 to 30 yr in the response of these glaciers to precipitation changes is implied.

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Early Flandrian land and sea-level changes in Lower Strathearn

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The morphological and stratigraphic studies reported here relate to early Flandrian relative sea-level changes in the carselands, or Postglacial raised estuarine flats, of Lower Strathearn. These data coupled with the study and dating of associated environmental changes by pollen and diatom analysis and radiocarbon assay, have enabled graphs of relative sea-level changes and land uplift to be constructed.

The carselands are backed by the Main Postglacial raised shoreline, whose local altitude is 9.8–10.2 m OD (ref. 1), and whose ^{14}C age has been determined elsewhere in east-central Scotland to be in the range 5,900–7,200 yr BP (refs 2–4). The carse deposits, consisting of estuarine silt and clay, vary in thickness from a thin veneer to well over 10 m, and are extensively underlain by a bed of terrestrial peat, which in turn rests on sandy estuarine deposits. The surface of the latter is shown by more than 250 boreholes sunk by the authors to form a staircase of buried steps separated from each other by distinct bluffs, the foot of each bluff representing a buried raised shoreline.

These morphological and stratigraphic relationships, which are broadly similar to those established in the Forth carselands^{5,6}, suggest the following sequence. A Lateglacial and early Flandrian phase of generally falling relative sea level was punctuated by stillstands and/or transgressive episodes resulting in the formation of what are now buried raised estuarine flats in descending order of age and altitude, the abandonment of the flats being followed by the growth of vegetation, including peat. Later, relative sea level rose again, causing the progressive burial of the peat-covered flats, in ascending order, by the carse deposits, and culminating in the formation of the extensive carseland surface visible today.

The 15 ^{14}C dates from six sites used in constructing the relative sea-level and land uplift curves (Figs 2 and 3) are listed in Table 1, and the locations of the sites are shown in Fig. 1. The data include two ^{14}C dates relating to the culmination of the Main Postglacial transgression at Glencarse⁷ (site 6), in the Carse of Gowrie, as this site is close to, and lies on the same Main Postglacial Shoreline isobase as, the Carey–Cordon area¹.

At Carey (site 1, Fig. 1) a 59 cm-thick bed of highly-compressed peat rests on medium sand, and is overlain by >6 m of carse deposits. The pollen record established by analysis of samples at 1–2 cm intervals throughout the peat and the upper 7 cm and lower 6 cm of underlying and overlying estuarine sediments contains no suggestion of any gaps in the depositional record. Although few unambiguous indicators of a salt-marsh environment are present, a reed-swamp succession may be suggested, especially at the basal transition, by the high frequencies of Gramineae pollen (up to 70% of 500 total land pollen). These are interpreted as reflecting the presence of *Phragmites*, of which there is also macroscopic evidence. The diatom assemblage of the basal 12 cm of carse deposits is dominated by several species of *Fragilaria*, averaging >70% of the total count (1,000 valves). In common with sites 2–5, diatoms are abundant only within and above the peat–carse transition, and the buried estuarine deposits contain only small numbers, which include both marine and brackish-water taxa. The base of the peat (altitude 3.2 m OD) at two different places along the same exposure has been dated at $9,640 \pm 140$ yr BP (I-2796)^{1,9} and $9,524 \pm 67$ yr BP (SRR-72)⁸, giving the approximate date of initiation of peat growth following the withdrawal of estuarine conditions (point 1a, Fig. 2). Pollen evidence confirms this date, with *Betula*, *Juniperus* and *Filipendula* all present in the basal peat samples, as has been found at Main Buried Beach sites in the Forth valley^{10,11}. As the Carey exposure is located close to the buried shoreline (altitude 3.2 m OD) the ^{14}C dates must relate closely to the abandonment of the latter, especially the one derived from the basal 1 cm of peat (I-2796). The Carey buried shoreline is believed to correlate with the Main Buried Shoreline of the Forth valley^{5,6}, which has been similarly dated at about 9,600 yr BP. (J. B. Sissons, personal communication). The top of the peat at Carey (present altitude 3.8 m OD) gave radiocarbon ages of $7,605 \pm 180$ yr BP (NPL-127)^{1,9} and $7,778 \pm 55$ yr BP (SRR-71)⁸, dating the onset of peat burial beneath the carse deposits (1b, Fig. 2). (Note that in ref. 9, the I-2796 date was erroneously recorded in yr BC instead of yr BP.)

At Innernethy (site 2) an 80 cm-thick peat bed rests on the silty sand of an extensive buried estuarine flat. The latter is next below the Carey feature in the staircase of buried flats, and site 2 is located adjacent to the buried shoreline (altitude 2.8 m OD). A 1 cm-thick peat band that occurs within the buried estuarine deposits, 18 cm below the base of the main peat bed, demonstrates that the buried shoreline was formed at the culmination of a transgression. The ^{14}C ages of this thin peat band (2a, Fig. 2; altitude 2.7 m OD) and the base of the main peat bed (2b; altitude 2.8 m OD) date this culmination to between $8,555 \pm 60$

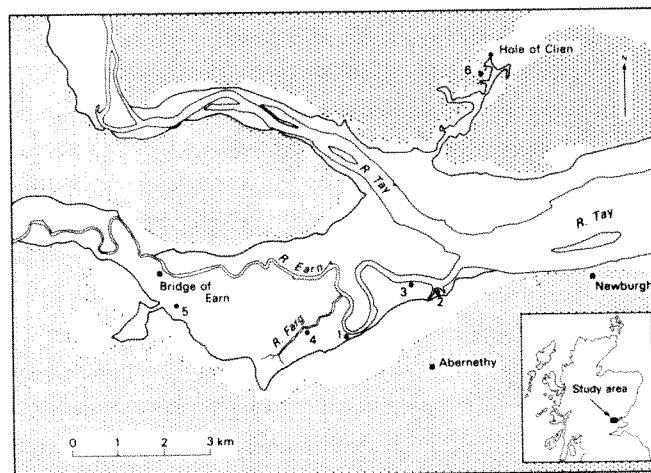


Fig. 1 Location of sites: 1, Carey; 2, Innernethy; 3, Cordon; 4, Culfargie; 5, Kintillo; 6, Glencarse. The heavy, continuous line represents the Main Postglacial Shoreline, marking the inner edge of the carselands. Land above 15 m OD is stippled.

(SRR-1399) and $8,505 \pm 50$ yr BP (SRR-1398). These dates are statistically indistinguishable at the 95% level from the only published date of $8,690 \pm 140$ yr BP (I-1839)^{4,6,10} relating to the Low Buried Beach in the Forth valley, with which the Innerneath buried flat should correlate by morphological analogy. This may imply that the Low Buried Shoreline was abandoned about 8,500 or 8,600 ^{14}C yr ago, rather than the 8,800 yr usually quoted^{6,13}, a possibility that is reinforced by the basal peat date at site 3. Apart from a brief appearance of *Chenopodiaceae* corresponding to the thin peat band within the buried estuarine deposits, the only apparent vegetational succession is at the peat-carse boundary, where a sequence of freshwater environments may be indicated before the onset of carse deposition (2c; present altitude 3.6 m OD) at about $7,530 \pm 50$ yr BP (SRR-1397). The diatom record in the carse deposits suggests marine and brackish water conditions, as indicated by *Paralia sulcata* (Ehrenberg) Cleve, *Rhaphoneis surirella* (Ehrenberg) Grunow ex Van Heurck and *R. amphiceros* (Ehrenberg) Ehrenberg¹².

At Cordon (site 3) a riverbank exposure at the front of the same buried flat on which site 2 is located shows a similar stratigraphy to that at site 1 (ref. 1). At both transitions the appearance of *Compositae*, *Chenopodiaceae*, *Plantago* sp., *Filipendula*, *Rosaceae*, *Typha angustifolia* and *Lemnaceae* may indicate a succession of salt-marsh and freshwater communities as suggested in the Forth valley¹¹. Marine and brackish water diatoms are prevalent within the basal carse assemblage with *Paralia sulcata* and *Rhaphoneis surirella* averaging over 50%. The basal peat date (3a, Fig. 2; altitude 2.8 m OD) is $8,370 \pm 45$ yr BP (SRR-1147), suggesting the removal of estuarine conditions slightly later than at site 2. This indicates abandonment of the frontal edge of the estuarine flat at a measurably later date than that of the shoreline 1 km to landward. The reappearance of estuarine conditions and initiation of peat burial (3b, Fig. 2; present altitude 3.2 m OD) occurred about $7,525 \pm 50$ yr BP (SRR-1394).

The basal peat dates at sites 1–3 all relate closely to the abandonment of two now-buried estuarine flats, the older and higher of which is the Carey flat. Three distinct buried steps occur above the Carey feature, and the rather thin peat on two of them has been dated at sites 4 and 5 (Fig. 1, Table 1). These higher buried steps are necessarily older than the lower ones already considered, yet the basal peat dates are much younger than at sites 1–3, and in fact differ little from the ages of the topmost peat layer at the latter sites. This suggests a lengthy

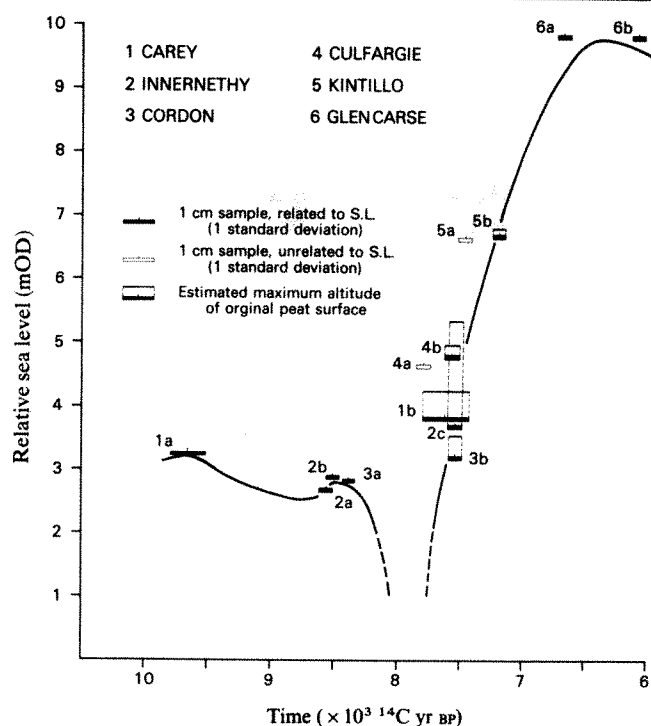


Fig. 2 Early Flandrian relative sea-level changes in the Carey-Cordon area, Lower Strathern. Horizontal bars extend one standard deviation either side of the ^{14}C dates.

hiatus between abandonment of the flats and the start of peat growth, and that the latter was a response to the rising groundwater level associated with the Main Postglacial transgression. The basal peat dates (4a and 5a, Fig. 2), although associated with marine transgression, cannot therefore be related to specific relative sea levels, and the higher buried flats remain undated.

Thin compacted peat at Culfargie (site 4) and Kintillo (site 5), 19 cm and 8 cm thick respectively, rests on fine sand and is overlain by 1.6 m and 3.5 m respectively of carse silty clay. Increased evidence of aquatic taxa is evident in the carse deposits at both sites and at Kintillo a sequence of freshwater and salt-marsh communities is suggested similar to that found at Cordon. Marine influence in the carse is also indicated by the abundance of *Paralia sulcata*. The basal peat dates at Culfargie

Table 1 Summary of details of radiocarbon-dated sites in Lower Strathern

Point no.	Site	Nat. grid ref.	Sample location	Altitude (m OD)	^{14}C age (yr BP) (uncorrected)	Lab. ref.
1a	Carey*	NO 1717 1710	Base of buried peat	3.19	$9,640 \pm 140$	I-2796
1b		NO 1747 1703	Base of buried peat	3.19	$9,524 \pm 67\ddagger$	SRR-72
2a		NO 1717 1710	Top of buried peat	3.78	$7,605 \pm 180$	NPL-127
2b		NO 1747 1703	Top of buried peat	3.78	$7,778 \pm 55\ddagger$	SRR-71
2a	Innerneath†	NO 1899 1783	Thin peat layer in silty sand beneath buried peat	2.66	$8,555 \pm 60$	SRR-1399
2b		NO 1899 1783	Base of buried peat	2.84	$8,505 \pm 50$	SRR-1398
2c		NO 1899 1783	Top of buried peat	3.64	$7,530 \pm 50$	SRR-1397
3a	Cordon*	NO 1845 1813	Base of buried peat	2.80	$8,370 \pm 45$	SRR-1147
3b		NO 1845 1813	Top of buried peat	3.16	$7,525 \pm 50$	SRR-1394
4a	Culfargie*	NO 1625 1717	Base of buried peat	4.60	$7,780 \pm 50$	SRR-1396
4b		NO 1625 1717	Top of buried peat	4.79	$7,555 \pm 50$	SRR-1395
5a	Kintillo†	NO 1348 1766	Base of buried peat	6.60	$7,465 \pm 55$	SRR-1401
5b		NO 1348 1766	Top of buried peat	6.68	$7,180 \pm 55$	SRR-1400
6a	Glencarse†	NO 2022 2256	Top of buried peat	9.52	$6,679 \pm 40$	SRR-1150
6b		NO 2022 2256	Base of surface peat	9.82	$6,083 \pm 40$	SRR-1151

* Riverbank exposure.

† Temporary excavation.

‡ These dates are not plotted in Fig. 2 because they were obtained on rather thick (4-cm) samples, and therefore relate less closely to the lithostratigraphic boundaries than the other dates, which were obtained from 1-cm thick samples.

(4a, Fig. 2; present altitude 4.6 m OD) and Kintillo (5a, present altitude 6.6 m OD), of $7,780 \pm 50$ yr BP (SRR-1396) and $7,465 \pm 55$ yr BP (SRR-1401) respectively, are verified by the high values for *Corylus/Myrica* pollen. Transgression of the peat surface occurred around $7,555 \pm 50$ yr BP (SRR-1395) at Culfargie (4b, Fig. 2; present altitude 4.8 m OD) and $7,180 \pm 55$ yr BP (SRR-1400) at Kintillo (5b, Fig. 2; present altitude 6.7 m OD).

In using peat-top dates to construct a relative sea-level curve account must be taken of the peat compaction that accompanied and followed burial by the carse deposits (compaction of the sandy sub-peat materials is assumed to have been negligible). Peat compaction at sites 1, 2 and 4 was estimated by comparing the mean dry bulk density value for an uncompacted monocotyledonous peat at Hole of Clie (NO 2034 2291), in the Carse of Gowrie, with similarly derived values for the buried peats in Lower Strathearn, on the assumption that the fossil peats, before burial, had a comparable bulk density to the surface peat, and that the amount of subsequent compaction is directly proportional to the increase in bulk density. To allow for variations in bulk density within the peat, the mean value for each site was derived from measurements carried out at vertical intervals of 1–4 cm through the peat. The estimated values for compaction at sites 1, 2 and 4 range from 40 to 68%, and in the absence of direct measurements at sites 3 and 5, an overall mean value of 52% was used at those sites. The estimated original altitudes of the peat surfaces at the time they were transgressed by the sea are shown in Fig. 2.

The age and altitude of the Main Postglacial Shoreline, marking the culmination of a major transgression, have been measured at Glencarse (site 6), where a 30 cm-thick layer of carse deposits is underlain and overlain by peat. The buried peat was transgressed (6a, Fig. 2) about $6,679 \pm 40$ yr BP (SRR-1150), and peat growth on the abandoned surface of the carse deposits (6b; altitude 9.8 m OD) was under way at $6,083 \pm 40$ yr BP (SRR-1151)⁷.

Two characteristics of the relative sea-level curve (Fig. 2) as yet unexplained are the transgressive nature depicted for the Carey buried shoreline (point 1a) and the fall of relative sea level between points 3a and 3b. The former is in line with the transgressive nature suggested for the correlative Main Buried Shoreline in the Forth area^{6,14}. The latter is suggested by a buried channel excavated to a few metres below OD near the present River Earn^{1,15}. This low point on the curve occurs between $8,370 \pm 45$ and $7,525 \pm 50$ yr BP, whereas the equivalent trough in the Forth curve is dated to between $8,690 \pm 140$ and $8,270 \pm 160$ yr BP (ref. 4). However, the discrepancy may be negligible when account is taken of the wider timespan delimited for this event by the available Lower Strathearn dates, and the possibility that the low relative sea level occupied a very brief timespan after $8,370 \pm 45$ yr BP.

The relative sea-level curve (Fig. 2) is primarily the net result of isostatic land uplift and eustatic sea level changes. A curve of land uplift (Fig. 3) for the period 9,500–6,500 yr BP was constructed by a similar method to that of Sissons and Brooks⁴.

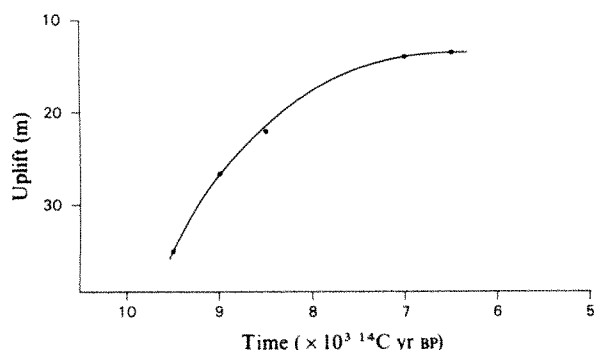


Fig. 3 Land uplift in the Carey-Cordon area between 9,500 and 6,500 yr BP. The graph shows total land uplift between each date and the present.

Land uplift at 500-yr intervals was calculated by adding the amount by which world sea level was below the present level at each date, according to a particular published eustatic curve, to the contemporary relative sea level in Lower Strathearn as shown in Fig. 2, and subtracting 2 m to compensate for the fact that the data in Fig. 2 relate to former estuarine shorelines and therefore to high water mark. Curves were drawn for all eight eustatic curves^{16–23} not rejected as unsuitable according to the criteria used by Sissons and Brooks, and the results averaged to produce Fig. 3. The form of the curve is strikingly similar to the Forth uplift curve, the magnitude of uplift being less in Lower Strathearn because of the greater distance from the centre of uplift.

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Effect of aluminium speciation on fish in dilute acidified waters

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Acidification of lakes and streams is a serious water quality problem in high elevation granitic ecosystems in the northeastern US^{1–3}. An important consequence of acidification is the mobilisation of aluminium from the edaphic to the aquatic environment^{3–5}. Elevated levels of aluminium may have serious ramifications for biological communities, particularly fish, inhabiting acidified aquatic systems^{5,6}. In this study, water quality data were collected from several acidified lakes and streams in the Adirondack region of New York state. The purpose of this investigation was to characterise aluminium chemistry in these acidified waters and to assess the relative toxicity of soluble aluminium species to fish. Aqueous aluminium speciation was found to be highly variable in Adirondack waters, and its effect on fish was also variable.

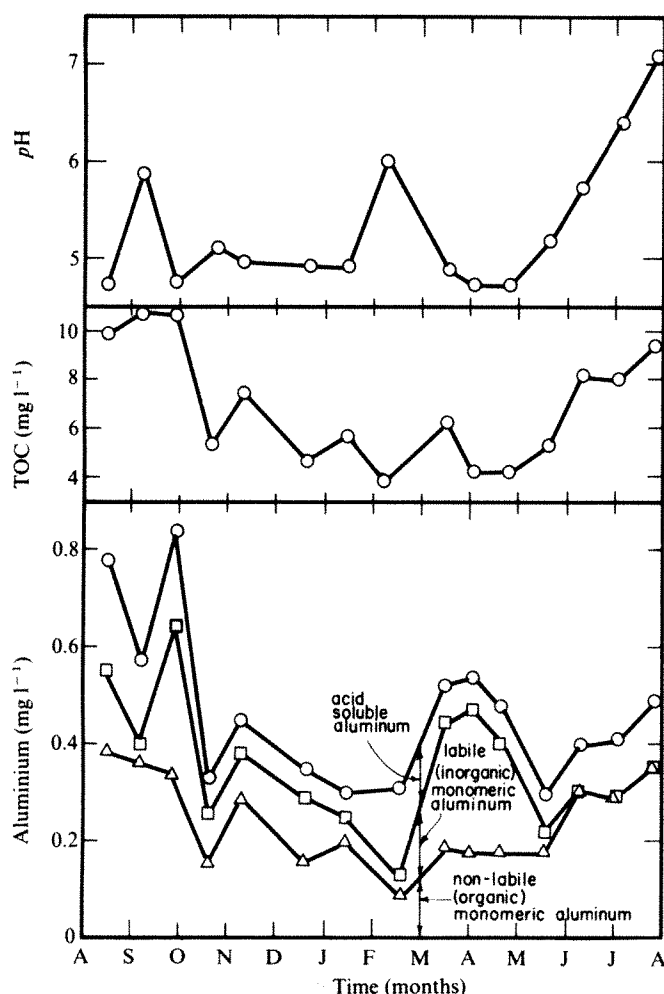


Fig. 1 Temporal changes in pH, total organic carbon (TOC) and aluminium forms for Canachagala Creek from August 1977 to August 1978. Low pH and high levels of labile monomeric (inorganic) aluminium were observed after rainfall (15 August, 27 September) and during snowmelt (15 March, 2 April and 23 April). Low water flow conditions produced elevated pH values and decreased labile monomeric (inorganic) aluminium levels. TOC and non-labile (organic) aluminium were generally high in the autumn and summer and low in the winter and spring. ○, Total aluminium; □, total monomeric aluminium; △, non-labile (organic) monomeric aluminium.

Three lakes in the southwestern part of the Adirondack Mountain Region were investigated—North Lake (74°55' W, 43°43' N; elevation 545 m); Big Moose Lake (74°50' W, 43°30' N; elevation 556 m); and Little Moose Lake (74°55' W, 43°42' N; elevation 545 m) and their tributaries were sampled over the course of a year. The edaphic, climatic and physiographical features of the Adirondack Region have been described by Schofield¹.

Three separate aluminium measurements were made on water samples using a colorimetric (ferron) method⁷. Measurements included total aluminium (samples acid digested before analysis), total monomeric aluminium (samples analysed without acid digestion) and non-labile monomeric aluminium (aluminium separated by a cation exchange chromatography technique and analysed as monomeric aluminium). From these measurements, the total aluminium content of each sample could be subdivided into three fractions. Non-labile (organic) monomeric aluminium was measured directly and included organically chelated aluminium. The second fraction, labile (inorganic) monomeric aluminium, was determined as the difference between total monomeric aluminium and non-labile (organic) monomeric aluminium. This fraction includes free

aluminium and aqueous inorganic complexes (fluoride, hydroxide and sulphate). The third fraction, acid soluble aluminium, was determined as total aluminium minus total monomeric aluminium and includes aluminium forms that require acid digestion before analysis (polymeric, colloidal, extremely stable organic and hydroxy organic complexes). pH was measured potentiometrically; fluoride was measured potentiometrically with a fluoride-selective ion electrode; sulphate was measured with the turbidimetric method and total organic carbon was measured by sample oxidation and IR detection of carbon dioxide using an organic carbon analyser. The labile inorganic fraction of monomeric aluminium was subfractionated using thermodynamic calculations into free (aquo) aluminium and fluoride, sulphate and hydroxide complexes of aluminium. Details of the analytical procedures and thermodynamic calculations used here are given by Driscoll⁸.

Some typical temporal changes in measured aluminium, pH and total organic carbon for Canachagala Creek, a tributary of North Lake, are given in Fig. 1. The relatively high concentrations of aluminium are representative of those found at the other sampling sites.

Table 1 Estimated 50% survival times of brook trout (*Salvelinus fontinalis*, Mitchill) in synthetic soft water solutions containing aluminium and aluminium complexing ligands

pH	Ligands added	Total aluminium concentration mg l ⁻¹	% Survival after 14 days		Estimated 50% survival time (h)	
			Mean	Range	Mean	Confidence limits
5.2	—	0.02	99	95–100	>500	—
	—	0.42	28	20–30	115	88–150
	Fluoride	0.50	45	35–45	202	136–302
4.4	Citrate	~0.5	96	87–100	>500	—
	—	0.01	95	80–100	>500	—
	—	0.48	42	27–53	256	213–306
4.4	Fluoride	0.47	71	67–80	472	354–629
	Citrate	~0.5	82	40–100	>500	—

50% survival times are maximum likelihood estimates based on a logistic model. Experiments were run for 14 days (336 h). Estimated survival times greater than the length of the experiment are based on extensions of the non-linear regression and are subject to large uncertainties. Thermodynamic calculations indicate that levels of ligands added should chelate nearly all aluminium present. Fluoride was added as NaF at 0.5 mg l⁻¹ F; citrate at Na₃H₅O₄ at 11.7 mg l⁻¹ C, at pH 4.4. At pH 4.4, all solutions were adjusted for equivalent Na levels (9.1 mg per l Na). The presence of citrate interferes with the measurement of aluminium. Aluminium levels are based on subsamples of water within experimental units. Each treatment had four replicates with 15–20 fish per replicate.

Periods of low pH were observed in the late summer and autumn after rainfall and during spring snowmelt. During the summer (1978) conditions were dry and pH levels consequently increased. During low pH conditions, total aluminium levels increased dramatically. However, the two fractions of monomeric aluminium (inorganic and organic) responded differently to changes in hydrogen ion activity. Increased levels of labile (inorganic) aluminium (Al³⁺ and aluminium fluoride, hydroxide and sulphate complexes) accounted for most of the response to decreasing stream pH and increasing flow. During high pH conditions, the inorganic fraction of aluminium was substantially reduced.

Variations in organically chelated aluminium seem to be independent of pH but are significantly correlated with total organic carbon measurements ($P < 0.0001$). In the tributaries studied, total organic carbon and non-labile (organically chelated) aluminium levels were low in the winter and spring and high in the summer and autumn. Therefore, the large fluctua-

tions in total aluminium concentration with pH in streams may be largely attributed to large fluctuations in the labile (inorganic) fraction of aluminium.

Aluminium forms strong complexes with hydroxide, fluoride, sulphate and dissolved organic ligands⁹⁻¹¹. All these potential aluminium ligands are present in the dilute acidified waters of the study lakes and streams. Results indicate that non-labile (organic) aluminium is generally the predominant fraction of aluminium in these waters. Thermodynamic computations indicate that fluoride complexes are generally the dominant inorganic aluminium species. Free (aquo) aluminium and aluminium hydroxide complexes were predicted to be present in lesser amounts and aluminium sulphate complexes were generally insignificant.

Elevated levels of aluminium in acidified surface waters may have serious effects on fish and other aquatic biota. Schofield and Trojnar⁶ indicated that levels of aluminium may be a primary factor limiting survival of trout stocked into acidified Adirondack lakes. However, speciation of aluminium was not considered in their study or in other studies of aluminum toxicity^{5,12}. It is generally accepted that complexation alters both distribution and toxicity of trace metals^{13,14}. Laboratory experiments from this study suggest that such conclusions are also applicable for aluminium.

Brook trout fry (*Salvelinus fontinalis*, Mitchell) were exposed in soft water to aluminium as the free ion and complexed with hydroxide, fluoride and/or citrate (Table 1). Fry were held in 8-l polyethylene units (20 fry at pH 5.2, 15 fry at pH 4.4 per unit, mean length 22–23 mm) with stock solutions changing at a rate of ~8 l per day. Stock solutions were prepared at least one day before use. Experimental units were aerated and temperatures ranged from 9 to 17 °C. Soft water was prepared with a Culligan reverse osmosis system using Ithaca city tap water and dechlorinated with a Barnstead organic removal filter. Basic cation concentrations in the softened water were 2–4 mg l⁻¹ Ca, 0.4–0.7 mg l⁻¹ Mg, 1.2–1.5 mg l⁻¹ Na and 0.2–0.4 mg l⁻¹ K. Total fluoride levels were less than 0.01 mg l⁻¹ F; sulphate levels were less than 5 mg l⁻¹ SO₄. Solutions were acidified with hydrochloric acid.

Treatments with excess fluoride or citrate decreased the toxicity of aluminium solutions (Table 1). Survival of brook trout fry after 14 days in treatment waters with 0.5 mg l⁻¹ Al and 11.7 mg l⁻¹ C citrate was 87% or greater at pH 5.2 and 40% or greater at pH 4.4, and not significantly different from control solutions at equivalent pH values. Additions of fluoride to acidified aluminium solutions also increased survival ($P < 0.001$ at pH 5.2, $P < 0.10$ at pH 4.4, Table 1). However, the mitigating effect of fluoride complexation on aluminium toxicity was less than that of organic complexation. Inorganic aluminium forms, therefore, seem to be the major species of concern with regard to aluminium toxicity in fish. As a result of reactions with hydroxide, aluminium toxicity to fish is pH-dependent. Schofield and Trojnar⁶ report increasing brook trout mortality and gill damage with increasing pH over the range 4.4–5.2 at fixed total aluminium levels (0.2–0.5 mg l⁻¹ Al).

Evaluation of aluminium speciation in acidified Adirondack waters indicates that organically complexed (non-labile monomeric) aluminium is the dominant form (Fig. 1). As complexation of aluminium with organic ligands seems to eliminate its toxicity, measurement of total aluminium concentrations may lead to a substantial overestimate of the potential aluminium-induced toxicity of acidified Adirondack waters. A comparison of mortalities of white sucker fry (*Catostomus commersoni*, Lacepede) in natural waters and in synthetic acidified water solutions containing aluminium (without additional complexing agents) supports this conclusion (Fig. 2).

For experiments with white sucker fry, 5-l polyethylene containers with 20 fry per unit were used. Approximately 1.5 l of stock solution was added to each experimental unit four times per day. Units were aerated and temperatures ranged from 17 to 19 °C. Two separate experiments were carried out at pH 5.0: 15–20 July 1978, mean length of fry at start of experiment

14.0 mm; and 7–12 August 1978, mean length of fry 18.7 mm. The sensitivity of white sucker fry to acidity and aluminium changes rapidly with age during the first few weeks after hatching (J. P. Baker, unpublished data). The fry become markedly less sensitive to low pH levels with time, but there may be a small increase in sensitivity to aluminium with increasing age.

Mortality rates of white sucker fry in three naturally acidified Adirondack waters (pH 5.0) appear to be determined by the levels of labile inorganic aluminium present (Fig. 2).

Water quality in dilute Adirondack waters is extremely variable, particularly with regard to organic carbon and hydrogen ion concentration. These variations have considerable effect on the aqueous aluminium chemistry, which in turn affects the survival of fish populations. Surges of labile (inorganic) aluminium into Adirondack streams during snowmelt and heavy rainfall are potentially lethal to eggs and fry. In addition, the ameliorating effect of organic complexation on aluminium toxicity suggests that lakes and streams with high organic carbon content may be suitable for successful fish production despite

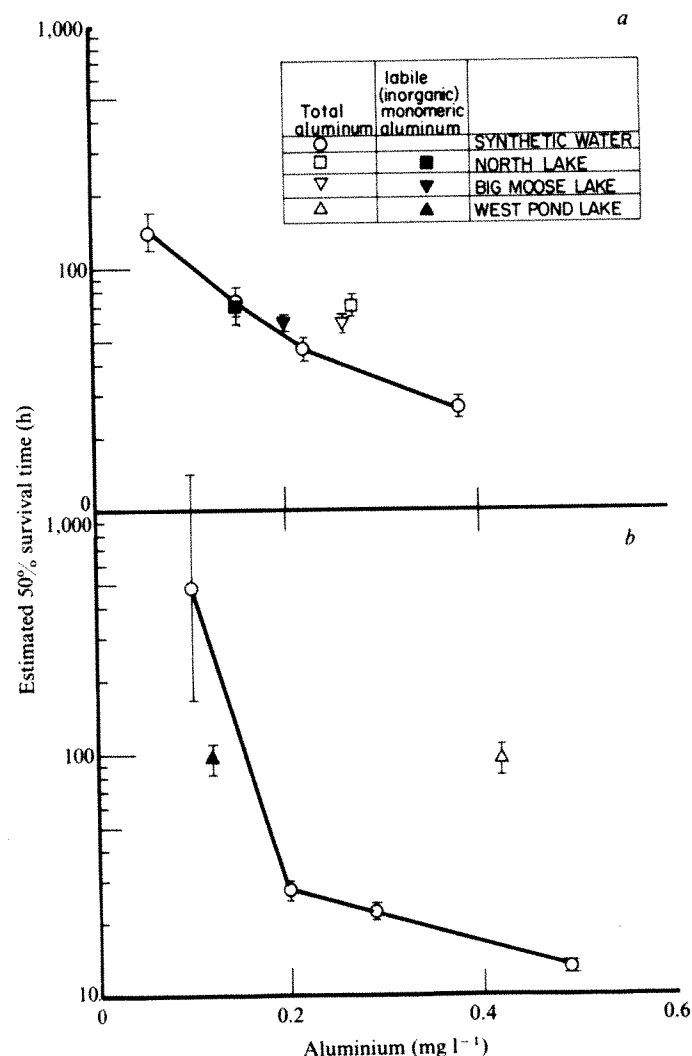


Fig. 2 Estimated 50% survival times (with 95% confidence intervals) of white sucker fry (*Catostomus commersoni*, Lacepede) at pH 5.0 in synthetic soft water solutions containing aluminium and in natural lake (Big Moose Lake, North Lake) and stream (West Pond Outlet, a tributary to Big Moose Lake) waters. 50% survival times are estimated as described in Table 1. Each value represents three or four replicate experiments with 18.7 mm fry with no aluminium added, survival was 95–100% after 5 days, and no 50% survival time was estimated. Mean fry length: a, 14.0 mm; b, 18.7 mm.

moderately low pH and high total aluminium levels. A detailed understanding of aluminium chemistry is essential to enable the effect of aluminium on the development of indigenous fish to be evaluated.

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Motion smear

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It is well known that the visual system summates signals over time, about 120 ms in daylight^{1,2}. Although this summation has the obvious advantage of enhancing visual sensitivity, it creates the potential problem of motion smear when viewing moving targets, whose images are also summated over time³. Here I report some measurements which reveal that provided the moving target is exposed for long enough to elicit a clear sensation of motion, the amount of smear is far less than may be expected. This suggests that the visual mechanisms which signal motion are also responsible for signalling a clear unsmear perception of the target in motion.

In addition to the threshold measurements of temporal summation it is also well established that super-threshold stimulation leads to a sensation which persists in vision for some time after the termination of the stimulus⁴, again, for about 120 ms in daylight^{5,6}. Perhaps the most direct measurements of the duration of this persistence were made by Ross and Hogben⁷ with dynamic random dot patterns, continuous sequences of brief flashes of light displayed at random positions on an oscilloscope face. Although only one dot is physically imaged on the

retina at any one time, an observer sees not just one, but a whole screen full of dots, in fact, all those that were displayed during the previous 120 ms. Clearly, under these circumstances, the neural sensations greatly outlast the duration of the visual stimulus.

This fact is of little consequence when viewing a stationary scene, but for moving targets, it poses the problem of motion smear. Objects travelling at even moderate speed cover a perceptible distance in 120 ms. If the same rules of visible persistence apply for moving scenes as for random dot patterns, we may expect moving objects to seem smeared and elongated in the direction of motion. This is illustrated by the photograph of Fig. 1, which was taken with a 125-ms shutter speed. Although the buildings all have good definition and contrast, the images of the people walking in the foreground are smeared beyond recognition. However, in natural viewing conditions, the images of moving objects always appear sharp and clearly defined, certainly very different from Fig. 1. Only very rapid image motion creates perceptible smear. Admittedly, with free viewing, the eyes will pursue a target of interest, but this does not solve the general problem, as it in turn introduces retinal motion of the background. What, then, are the mechanisms of vision that prevent motion smear?

Although this problem is fundamental to the understanding of dynamic visual perception, it has received surprisingly little attention. Many measurements have been made on the effect of image motion on acuity^{8,9}, but these do not necessarily reflect the more subjective perceptual qualities of smear or apparent elongation. Two investigations which do bear on this question report that the clarity and veridical perception of a target in

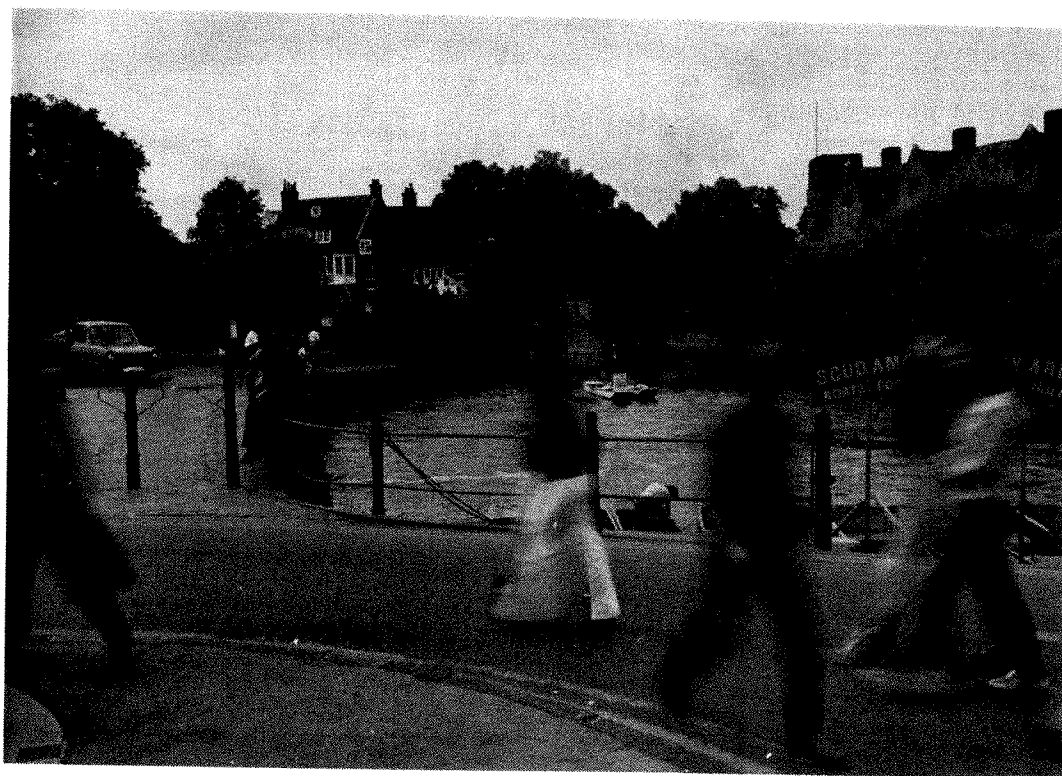


Fig 1 Vision through a 125-ms temporal window. Although much evidence suggest that the visual system integrates light for 125 ms, our every day perceptions differ markedly from a photograph taken with this integration period (shutter speed).

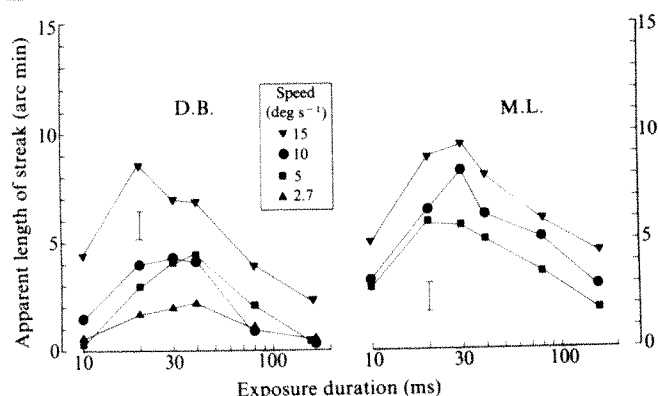


Fig. 2 The apparent length of the motion streak of dots moving along a constant linear trajectory, as a function of exposure duration. For durations of up to ~30 ms, the apparent length increases with increasing duration, as may be expected were light to be integrated over this period. However, at durations of longer than 30 ms, the apparent length decreases with increasing durations—the further the dots travel, the shorter they appear. The error bars each represent 2 standard errors, averaged over all conditions.

motion are enhanced by the prior and post exposure in locations at either end of the motion sequence^{10,11}. Here I show that even without pre- and post-viewing of stationary targets (which, given the filtering properties of vision, alters the effective visual velocity¹²), the amount of apparent elongation or smear is far less than might be expected on the basis of visual persistence⁷.

With a simple matching technique, I have measured motion smear as a function of the duration of exposure to the motion sequence. The measurements were made by matching the length of a short stationary line to the apparent length of dots in an array of motion, using a multiple interleaved staircase procedure under computer supervision¹³. The observer saw a short sequence of moving dots, immediately followed by a continuously visible stationary line. By pressing the appropriate button, he indicated which of the two stimuli seemed to be longer. Depending on his response, the length of the line was either incremented or decremented on the next trial of that condition. The apparent length, taken as the mean of the previous 15 trials, was measured separately three times to give a rough estimate of measurement error. The stimulus pattern was an array of 100 dots in random locations, intensified on an oscilloscope face (p15 phosphor) to a contrast 100 times their visibility threshold against a uniform background luminance of 30 cd m⁻². The dots were caused to move by displacing them successively at 5-ms intervals between each brief (4 μ s) intensification, on some trials to the left and on some to the right, the two directions being presented in random order. Short presentations of unpredictable direction of motion precluded the possibility of pursuit eye movements¹⁴. The screen was vignettted down to a 5° diameter circle by a mask of the same mean luminance placed outside the observer's field of focus, so that the dots faded smoothly out of view. Measurements are reported for two observers, D.B. and M.L., but the effect has been verified qualitatively with many observers, and indeed, can be readily verified by anyone with access to basic visual display facilities.

The results are shown in Fig. 2. Dots exposed for long durations, which travel over a larger retinal region, may be expected to seem more elongated than those exposed only briefly. Indeed, if all the light impinging on the retina were seen simultaneously, as with the dynamic random dot patterns, the perceived length of the streak should be equal to the distance covered by the moving dot. However, this is far from being the case. At short durations (up to 20–30 ms) the amount of motion smear does increase with duration, but at longer durations, the perceived length actually decreases with duration—the further the dots travel, the shorter they seem. Whereas with brief presentation times one sees an array of almost stationary line segments, at longer durations the stimuli are unquestionably small dots in motion, with no hint of elongation along their

trajectory, except when moving at the fastest speed, 15 deg s⁻¹. However, even at this speed the apparent length was only 2 or 4 arc min (for D.B. and M.L.), compared with the 100 arc min the spot traverses during the 120 ms of 'visual persistence'.

Not only was there less elongation of the dots, but also the sensation of motion became much stronger at longer durations. All the stimuli seemed to move to some extent, but with brief exposure times the sensation was quite weak. The non-occurrence of motion smear seems to be linked to the perception of smooth motion. Only when the motion seemed smooth and realistic was the target seen as a small, sharp, unsmearred dot. This suggests, that, contrary to the currently favoured idea of separate analysis of pattern and motion^{15,16}, the mechanisms of vision specialised to detect motion are also responsible for the analysis of the spatial form of the target in motion. Brief glimpses of a motion sequence fail to activate these mechanisms, activating instead mechanisms which, like a camera of long exposure, signal motion smear. A more detailed account, explaining these and other similar results in terms of the known spatial and temporal tuning properties of visual mechanisms, is in preparation.

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Mastectomy and mammary glands in reproductive control in the goat

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Little attention has been paid to the role of the mammary glands in reproduction apart from the suppression of reproductive activity during lactation in some species. A hint of greater involvement, however, stems from two observations in the goat. First, Linzell¹ reported that while developing his technique of transplanting mammary glands to other sites in the body in order to gain access to the mammary artery, the incidence of reproductive disturbances (infertility, abortion and apparently inexplicable maternal death at parturition) seemed greater in goats which had lost mammary tissue post-operatively, or which retained only one mammary gland, than in others in the herd and second, the mammary glands may significantly influence the concentration of a hormone in the general circulation²⁻⁵. Moreover, in some marsupials there is evidence that the mammary glands are involved in the control of reproduction, for example, denervation of the mammary glands during lactation initiates development of the diapausing embryo⁶. In view of the importance of reproductive integration in the artificial control of fertility, we have followed up these clues by investigating the effects of mastectomy in the goat, a species with a seasonal, rather than a lactational, anoestrus. We report here that the oestrous cycle was markedly disturbed by mastectomy and that fertility, and possibly also the length of gestation, seemed to be affected.

Table 1 Effects of mastectomy on oestrous cycles of goats approximately 1 yr after operation (s.e.).

	Length of oestrous cycle* (days)				Duration of behavioural oestrus (h)	
	1st	2nd	3rd	4th	1st	Subsequent
Intact controls (5)	22.0 ± 1.48	21.6 ± 0.25	21.4 ± 0.25		approx. 30	approx. 30
Operated controls (5)	24.4 ± 0.51	21.0 ± 0.01	21.6 ± 0.25		approx. 30	approx. 30
Mastectomised (5‡)	8.4 ± 0.40	13.0 ± 2.45	17.0 ± 1.41	14.3 ± 2.21	115 ± 13.9	approx. 30
	$P < 0.001$	$P < 0.01$	$P < 0.05§$			

Values given are means ± s.e. P values are with respect to both intact and operated groups except that marked §, $P < 0.01$ compared with the operated controls.

* First day behavioural oestrus observed = day 1 of cycle.

† Between first and second oestrus, and so on.

‡ One goat developed hepatitis during second cycle and had to be killed.

Both mammary glands were removed, under halothane anaesthesia, from five goats aged 9 months. The operations were done in early December (that is during the breeding season) 3–7 days after the last observed oestrus. No sham operation could be devised to mimic closely the full procedure of mastectomy. Therefore, the operated animals were compared with two control groups—five intact goats and five which had been subjected to udder surgery (such as ligation of blood vessels crossing between the two glands⁷) akin to that completed by mastectomy but in which neither the main nerves nor major blood vessels had been divided. Although in young virgin goats mastectomy is a relatively minor surgical procedure, the results obtained in the first breeding season are not presented to avoid the possibility that the effects observed might be attributed to nonspecific effects of surgery. However, it is interesting that one goat never returned to oestrus for mating and in two others fertility seemed to be reduced.

In the second breeding season, approximately 1 yr after operation, the goats were observed several times each day for the signs of behavioural oestrus (bleating, restlessness and tail-wagging). The mastectomised goats showed the first signs of behavioural oestrus during a period in the autumn indistinguishable from that of other goats in the herd; in other words they emerged from seasonal anoestrus at the same time. However, major differences then became apparent. Thus the first oestrus of the season lasted 3–6 days in the mastectomised goats compared with approximately 30 h in the controls. Moreover, the first cycle (between the onset of the first and the second oestrus) was significantly shorter in the mastectomised animals (mean 8.4 days) than in either the intact (22 days) or operated (24.4 days) controls. Subsequent cycles were also significantly shorter, although the duration of behavioural oestrus was not apparently affected (Table 1). The mastectomised goats were all mated at the second oestrus but all returned to oestrus at least twice more (two, two, three and four times, respectively, in the four surviving goats (Table 1), even though they were mated each time. By contrast, the controls that were mated first (with the same males) at the fourth oestrus showed no further cycles. Moreover, in the rest of the herd mated at the first to fourth oestrus of the season, only four out of 80 matings (in two seasons) did not result in conception at the first attempt.

The gestation period (in days) of the four goats that became pregnant in their first season (number of young delivered in parentheses) were: 146 (2), 146 (1), 146 (1) and 147 (1), and in the second season: 143 (2), 147 (1), 147 (2) and 148 (2). Because the number of young carried seems to affect gestation period, comparison with controls is difficult. Nevertheless, for those bearing one kid the gestation periods were all shorter (by a median of 4.5 days) than the median of 151 days for goats in the herd⁸; similarly, for those bearing two the gestation periods were all shorter (by a median of 3.5 days) than the median of 150 days⁸. All young were delivered alive.

These results indicate that mastectomy had a pronounced effect on oestrous cycles in the goat; in addition, fertility, and possibly also the duration of pregnancy, seemed to be affected. Further experiments are in progress, but it would seem that

there is an alteration in the 'fine-tuning' of reproductive processes. It can be argued that it is the presence of mammary tissue rather than lactation which is of major importance in the effects described because there seems to be no difference between lactating and non-lactating intact goats in terms of the periodicity of oestrous cycles or of fertility (our unpublished observations).

Although Linzell¹ found no apparent effects of mastectomy on reproduction in the guinea pig, the strategies of reproductive control used by mammals are so diverse that it remains to be seen whether reproductive difficulties supervene in individuals of other species which suffer loss of mammary glands or tissue, either surgically as in women with mammary tumours, or following infection as in mastitis in farm animals.

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Evidence for selection following perturbation of allozyme frequencies in a natural population of *Drosophila*

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There is now ample evidence for the ubiquity of genetic variation at allozyme loci in many species^{1,2}, but the extent to which this variation is actively maintained by natural selection remains unresolved. Although various approaches have suggested the importance of selection at some allozyme loci in laboratory populations^{3–6}, to be fully convincing, evidence for natural selection must finally come from studies on natural populations. Inevitably, this will entail an understanding of and the potential to manipulate both the ecology and genetics of the population. Because sufficient is known of the ecology, breeding site, nutritional requirements and field behaviour of at least some species of the cactophilic *Drosophila*, we^{7–9} and others¹⁰ have chosen them for experimental evaluation of the forces operating to maintain genetic variation. If genetic variation at some locus is being actively maintained by natural selection, the action of this selection should be detectable following artificial change in gene frequencies produced either by adding certain genotypes to the population, or by removing them from it. Jones and Parkin¹¹ have reported unsuccessful attempts to detect selection by such

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perturbation experiments in *Cepaea* and *Drosophila pseudo-obscura*, while Halkka *et al.*¹² inferred strong natural selection at a locus controlling colour polymorphism from exchange of individuals of *Philaenus spumarius* between two island populations. We report here results for the simultaneous perturbation of gene frequencies at three allozyme loci in *Drosophila buzzatii*. The changes in gene frequency following this perturbation provide strong evidence for selection, and for differential selection among the three loci.

The loci chosen for perturbation were *esterase-2* (*Est-2*), *pyranosidase* (*Pyr*) and *alcohol dehydrogenase-1* (*Adh-1*), which are on different chromosomes. The only known chromosome inversions are on the second chromosome¹³, and although *Est-2* is also on this chromosome, its precise relationship to the inversion has not been determined. The alleles whose frequencies were increased were the comparatively rare *Est-2*^c and *Pyr*^b, and the intermediate frequency *Adh-1*^c. At one population in the Hunter Valley, NSW, Australia (locality 5 of ref. 7), wild flies have been collected monthly over a 4-yr period to study temporal changes in gene frequencies. From wild females collected during this temporal study, 37 isofemale lines were made simultaneously homozygous for *Est-2*^c, *Pyr*^b and *Adh-1*^c. These lines were crossed in pairs, then double-crossed and progeny of the latter used to initiate two duplicate population cages.

The population that was perturbed inhabits an isolated clump of *Opuntia inermis* comprising about 40 large plants in an area about 50 × 20 m, and is located some 7 km from the temporal study site. The surrounding area is cultivated or open grazing land, and the nearest *O. inermis* is some 3 km distant. Four estimates of gene frequencies in this population (times 1–4, Fig. 1) were made over 8 weeks before perturbation began. The first

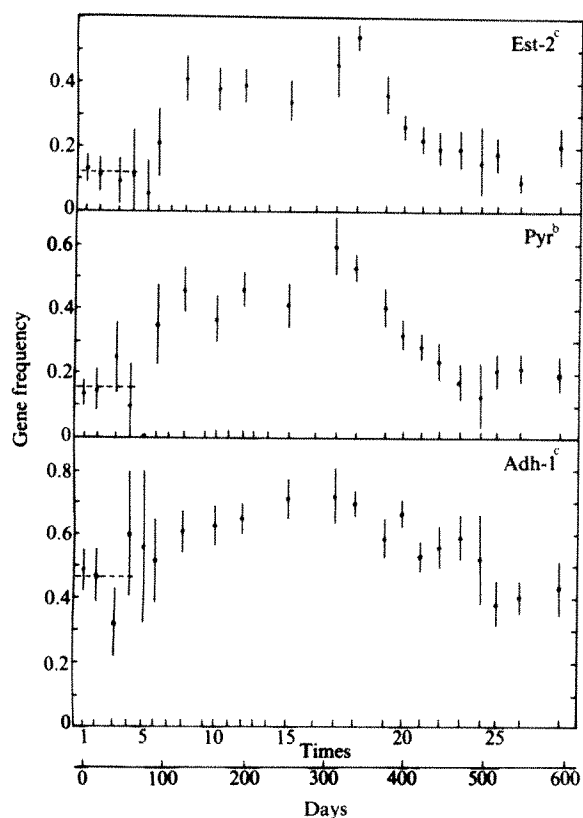


Fig. 1 Changes in gene frequency of *Est-2*^c, *Pyr*^b and *Adh-1*^c during perturbation during times 4–18, and following cessation of perturbation. The dotted line through times 1–4 is the pre-perturbation weighted average gene frequency. Times refer to the sequential number given to each collection and/or release. The gene frequencies plotted are those for non-release flies only, all collected flies that were homozygous *Est-2*^c, *Pyr*^b and *Adh-1*^c being excluded.

Table 1 Post-perturbation phase weighted average migration rates (*m*), range of estimated migration rates over the three loci and heterogeneity χ^2 for different periods

Times	<i>m</i>	Range	χ^2	
18–23	0.827	0.443–0.941	6.671	<i>P</i> < 0.05
18–24	0.951	0.742–1.042	0.898	
18–25	0.883	0.841–1.328	5.412	<i>P</i> < 0.10
18–26	0.990	0.821–1.217	9.162	<i>P</i> < 0.05
18–27	0.855	0.794–1.190	3.867	

release was done at time 4 after collection of flies for gene frequency estimation.

Perturbation was done in two ways. By continuous egg sampling from the homozygous laboratory cages, replicate cages were produced containing all developmental stages from eggs to newly enclosed adults. Two of these were set up in the field every 2–3 weeks, protected against predatory mice and insects and from the direct effects of sun and rain, but with an opening in the lid allowing eclosing adults to escape. Adults entered the population from these cages over a period of 3–6 weeks, depending on the season. In addition, we exploited knowledge of the breeding site of the species. It was thought that, by allowing the immature stages to feed and develop in their natural environment, they might be better adapted to enter the natural population than cage-reared adults. Accordingly, each time that cages were put out in the field, a thick suspension of eggs and young larvae from one to four of the most recently added cage cups was injected into as many rotting cladodes as could be located.

Thirteen such releases were made over 253 d (at times 4–17, excluding time 10). Gene frequency increased significantly at all three loci, but the pattern of change differed for *Est-2*^c and *Pyr*^b compared with *Adh-1*^c. The former initially increased rapidly, remained approximately constant for about 100 d during autumn–winter, and increased again in spring. In contrast, *Adh-1*^c increased steadily through autumn–winter. Selective effects may be implicated as *Adh-1*^c tends to be at higher frequency in the southern part of the species distribution⁷, and its frequency increased among survivors in cold shock experiments (A. W. Watt, personal communication).

After time 18, gene frequencies decreased to approximately pre-perturbation values, but again the patterns of change differed for *Adh-1*^c compared with *Est-2*^c and *Pyr*^b. The latter decreased steadily, but *Adh-1*^c showed a slower and more irregular decrease.

The observed increases in gene frequency during the perturbation phase must have been due to migration into the population of release flies, whereas the decreases in the post-perturbation phase could have been due only to selection or to migration from other populations. For any given time period, if changes in gene frequency were due only to migration, the estimated migration rates for each locus should not be significantly different. However, if there were any differential selection among loci, this could be detected as significant heterogeneity among the estimated migration rates. For each of the perturbation and post-perturbation phases, these migration rates and χ^2 tests of heterogeneity have been estimated¹⁴. During perturbation, migrants were assumed to be release flies only, and the estimated migration rates were not heterogeneous. Weighted average rates for different periods were: pre-perturbation to time 18, 0.458; times 4–18, 0.473; times 5–18, 0.523; times 6–18, 0.375. Thus, some 40–50% of gametes entering the population came from release flies. For post-perturbation, gene frequencies in any migrants are unknown, but were assumed equal to the pre-perturbation weighted average frequencies. Gene frequencies in any migrants from nearby populations are unlikely to be very different; for example, average frequencies in the temporal study population were *Est-2*^c 0.113, *Pyr*^b 0.149, *Adh-1*^c 0.505, as compared with the pre-perturbation weighted average frequencies of 0.120, 0.152 and 0.462, respectively. For the post-perturbation phase, weighted average migration rates

(m), the range of estimated migration rates for each locus and heterogeneity χ^2 for different periods were as shown in Table 1. Given the significant heterogeneity among migration rates and the extraordinarily high rates necessary to account for the changes in gene frequency, migration is not a sufficient explanation for the decreases in gene frequency. Therefore, the post-perturbation decrease of gene frequencies to the pre-perturbation values must be ascribed to natural selection.

We cannot argue from these data that selection has acted directly at the allozyme loci, and two arguments could be raised to suggest that selection may have been acting against chromosome regions marked by these loci. First, although 37 separate lines contributed to the release population, so that its genotype at other than the three loci and closely linked regions should reflect the variability in the natural population from which the lines derived, this latter population was not the one that was perturbed. However, as noted previously, it was only 7 km distant, average gene frequencies for polymorphic allozyme loci were very similar, and it was presumably subject to similar pressures of natural selection. Second, the wild females used to produce the homozygous lines were collected over 6 months, and the time from the middle of this period to the last release at time 17 was 21 months. Adaptation to laboratory conditions during this time may have been such that the post-perturbation effect was one of selection against laboratory-

adapted genomes. Although this is possible, it must be emphasised that during the perturbation phase these genomes were successfully incorporated into the wild population and at least partly recombined with wild genomes. Also, alleles favoured in the laboratory environment but deleterious in the wild should have been largely eliminated during this phase. Finally, there must have been differential selection among the three loci (or among chromosome regions marked by them) to account for the observed heterogeneity in estimated migration rates.

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Anaesthetics increase light emission from aequorin at constant ionised calcium

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The isolation by Shimomura, Johnson and Saiga¹ of a protein, aequorin, that emits light in the presence of micromolar concentrations of ionised calcium opened up new possibilities for the study of ionised calcium inside cells²⁻⁵. It is a relatively simple matter to introduce aequorin into large cells by microinjection, and the rate of light emission gives a direct measure of intracellular free calcium. During an investigation into the action of certain anaesthetics on squid axons, we noticed that these agents always increase the light output from intracellular aequorin. Subsequent analysis has now revealed that this does not result from a rise in ionised calcium inside the axon but seems to reflect a direct effect of the anaesthetic agent on the aequorin molecule. The agents studied all produce greater activation of the light emitting reaction at a constant level of ionised calcium. These rather simple observations have several important biological implications: (1) aequorin might be an interesting model system for studying interaction between anaesthetics and proteins, (2) other Ca-sensitive proteins might behave like aequorin, their affinity for calcium being subject to modulation, and (3) such modulation of endogenous Ca-binding proteins might contribute to the mechanism of anaesthesia.

Figure 1 shows the simultaneous measurement of aequorin light output and calcium efflux from a squid axon. Application of urethane produces a reversible increase in light output which is largely independent of the calcium content of the sea water. The most obvious explanation for this observation is that urethane has, in some way, raised the intracellular ionised calcium concentration, possibly by effecting release of bound calcium. The efflux data of Fig. 1 provide no evidence for such an effect because a rise in ionised calcium should lead to an increase in calcium efflux and the efflux does not change during exposure to urethane.

An alternative explanation consistent with Fig. 1 is that the anaesthetic is not altering intracellular ionised calcium but is

acting directly on the aequorin molecule. This possibility was investigated by introducing aequorin into a 500- μ m diameter porous cellulose acetate tube⁶ and superfusing the trapped aequorin with 0.5 M KCl solution containing 10 mM phosphate, pH 7.2, and a variety of Ca-EGTA buffers. Figure 2 shows that in this *in vitro* assay system urethane still increases light output even when ionised calcium is held constant by a relatively high concentration of calcium buffer. On a log-log plot the effect of urethane is to shift the relationship between light output and ionised calcium to the left without change in slope. As the total light output—assayed by injection of aequorin into a 0.5 M solution of calcium acetate—is not obviously different within the experimental error of about $\pm 5\%$ in the presence of up to

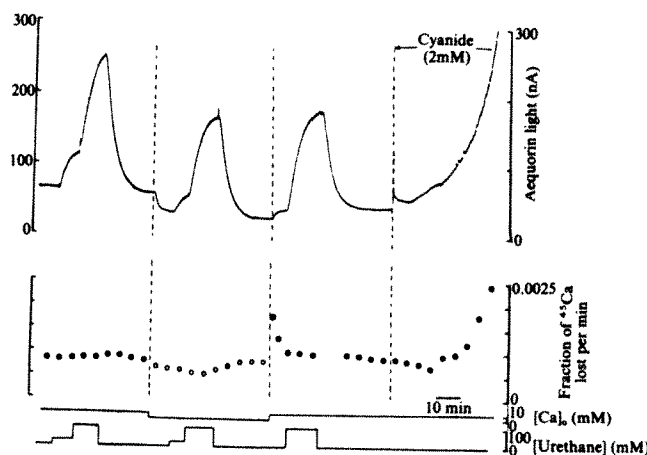


Fig. 1 Simultaneous measurement of aequorin light output (upper trace) and ^{45}Ca efflux (lower trace) during exposure of a cleaned axon of *Loligo forbesi* to urethane and cyanide. Axon 830 μ m; temperature 20 °C. Axon immersed in artificial sea water containing (mM): NaCl, 400; MgCl_2 , 100; CaCl_2 , 10; KCl, 10; NaHCO_3 , 2.5. Calcium was removed for the period indicated. Urethane and cyanide were added to the sea water at the times shown. Note that during the period of urethane application, although the light output increased, calcium efflux was little affected whereas during application of cyanide both light emission and calcium efflux increased roughly in parallel.

200 mM urethane, these observations indicate that urethane is increasing the affinity of aequorin for calcium.

Urethane affects aequorin in the same concentration range as that required to produce anaesthesia. Half-maximal inhibition of the sodium current in squid axons requires about 100 mM urethane⁷. Clear effects of urethane on aequorin can be seen at 20 mM and the increase in rate of light emission is half-maximal also at about 100 mM. Essentially similar results have been obtained with several other anaesthetic agents (Fig. 3). Diethyl ether (100 mM), chloroform (50 mM), ethanol (100 mM), procaine (25 mM), tetracaine (2.5 mM) and althesin increase the light emission by aequorin at constant buffered concentrations of ionised calcium in the physiological range. However, our findings are at variance with those of Kamaya *et al.*⁸, who reported a reduction in calcium-induced light emission from aequorin exposed to a variety of anaesthetic agents. The reason for this difference is not clear. We have only seen inhibition of light output at very high concentrations of urethane and ethanol.

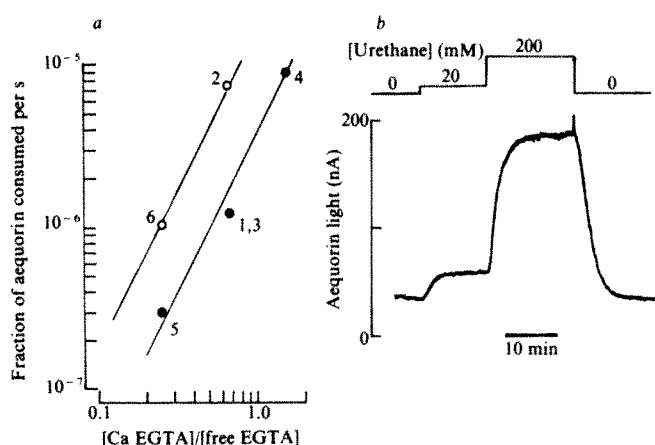


Fig. 2 Effect of urethane on aequorin light output *in vitro*. Continuous measurement of light output from a single sample of aequorin enclosed in a porous cellulose acetate capillary and superfused with test solutions of composition (mM): KCl, 500, potassium phosphate, pH 7.1, 20; Ca-EGTA buffer, 5. *a*, Shows a log-log plot of light output against ionised calcium in the absence (●) and presence (○) of 200 mM urethane. Points were obtained in the order indicated. *b*, Shows light output for a sample of aequorin exposed to 20 and 200 mM urethane in the presence of a Ca-EGTA buffer containing 4 Ca: 10 EGTA. Temperature 20 °C.

These findings are of interest for two reasons. First, we have demonstrated a clear effect of a variety of anaesthetic agents on a well defined soluble protein; this raises once again the possibility that general anaesthesia may result from a direct interaction between anaesthetic and protein in the nervous system. Second, it is possible that other Ca-binding proteins behave like aequorin. It has long been recognised that many intracellular processes are regulated by Ca^{2+} ions. The first to be understood at the molecular level was the regulation of muscular contraction, where the binding of calcium to a low molecular weight protein, troponin C, produces a conformational change that leads to activation of myosin ATPase⁹. More recently, evidence has accumulated that troponin C is only one example of a whole class of Ca-binding proteins (calmodulins) that confer calcium sensitivity on intracellular processes¹⁰⁻¹². The characteristic features of these molecules are a relatively low molecular weight, more than one Ca-binding site and a large conformational change associated with uptake and release of calcium. The resemblance to aequorin is striking and it seems important to establish whether the affinity of these molecules for calcium may be subject to physiological and pharmacological modulation in a manner analogous to that which we have described for aequorin. This could add a further dimension to the already

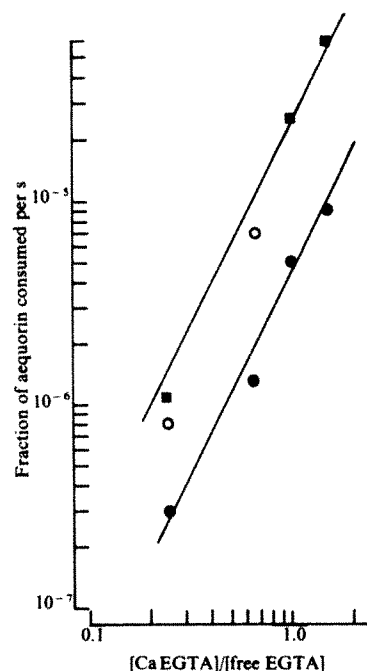


Fig. 3 Effects of diethyl ether (200 mM, ○) and tetracaine (2.5 mM, ■) on aequorin light output *in vitro*, and light output in the absence of anaesthetic (●). Data from two separate experiments carried out as described in Fig. 2a legend.

complex field of calcium regulation, because not only could reaction rates be altered by changes in free calcium, but they could also be controlled at constant ionised calcium by modifying the affinity of their respective binding sites (calmodulins) for calcium.

To return to the specific case of anaesthesia, anything that increases the permeability of nerve cell membranes to K^+ or Cl^- will render the cells less easy to excite. Both K^+ and Cl^- permeability systems exist in calcium-sensitive forms^{13,14} and Krnjevic¹⁵ has suggested that anaesthetics may activate these systems by producing a rise in intracellular free calcium. The present results suggest an alternative mechanism: anaesthetics may make these calcium-dependent permeability systems more sensitive to calcium, and so reduce excitability without the need for any change in intracellular ionised calcium. This may explain the recent observations of Simons¹⁶ that propranolol increases potassium permeability of human erythrocyte ghosts in which the internal calcium is stabilised by EGTA buffers. In addition to permeability effects, the other calcium-sensitive processes in nerve cells that merit close examination are transmitter release, Ca ATPase and the regulation of cyclic nucleotide levels.

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Potassium channels in nodal and internodal axonal membrane of mammalian myelinated fibres

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Horakova *et al.*¹ were the first to observe that the phase of late outward current carried by potassium ions in frog and squid nerve is virtually absent in voltage-clamped rat nodes of Ranvier. This observation has been recently confirmed by Chiu *et al.*² in rabbit nodes of Ranvier, suggesting that the nodal membrane in the mammal generally has few if any potassium channels. The present voltage-clamp experiments show that large potassium currents can, however, be produced in normal rabbit nodes of Ranvier by acute treatment designed to loosen the myelin from the axonal membrane. From this we conclude that whereas potassium channels are absent in the mammalian nodal membrane, they are normally present in the internodal axonal membrane at least in the paranodal region.

Figure 1Ai shows a series of ionic currents (after subtraction of a linear leakage current) obtained in a rabbit node of Ranvier. Starting at an initial holding potential of -80 mV the membrane was clamped to a series of equally spaced potentials in the range -72.5 mV to $+62.5$ mV. The early sodium current is clear in the records whereas there is very little late outward current. Such outward current as was present was quite insensitive to externally applied tetraethylammonium ion (TEA). Various procedures were then applied to loosen the myelin. Typically, the preparation was first exposed to collagenase (Sigma) for 2–10 min to loosen connective tissue. The solution bathing the node was then alternated between normal Locke solution and various modified Locke solutions designed to produce volume changes in the nerve. Three such solutions were used: a Locke solution in which half of the sodium was replaced by potassium, a hypertonic solution produced by adding 0.5 M sucrose to Locke, and a hypotonic solution obtained by diluting Locke with an equal amount of water. These procedures, undertaken in the hope of loosening the axonal membrane from the investing myelins, were repeated several times until a sudden and dramatic increase in the outward current was observed (Fig. 1Bi). Addition of tetrodotoxin (to block the sodium current) left only the outward current (Fig. 2A). External application of 20 mM TEA at this stage usually reversibly reduced the outward current by about 30%. However, when the solution in the end pools was changed from a potassium chloride solution to a caesium chloride (80 mM) and TEA chloride (80 mM) solution the late outward current decreased by about 70% in 10 min (Fig. 2C).

Concomitant with this large increase in outward current was a marked change in the shape of the transient capacity current. Whereas before treatment the initial capacity transient settled quickly in a fraction of a millisecond to a steady state leakage value (first few sampling points after the pulse in Fig. 1Aii), after treatment an additional slow current transient developed with a time constant of 0.5–1.5 ms (Fig. 1Bii). Experiments using hyperpolarising pulses to different potentials between -90 mV and -125 mV showed that this slow current was a linear capacity current. Thus, the time constant of this slow current measured during the pulse was voltage-independent; and it was about the same size as that measured after the end of the pulse. Furthermore, the time integral of this slow current (after subtraction of a steady leakage) obtained during the pulse (that is, the total charge displaced during the on-response) varied linearly with voltage, and roughly equalled that obtained in the off-response following the pulse. We believe that this slow capacity current

occurred because the 'myelin loosening' treatment had exposed a large amount of new axonal membrane previously covered by the myelin, and that the capacity of this newly exposed membrane was now being charged by the voltage-clamp through a large series resistance. For the particular node in Fig. 1, the

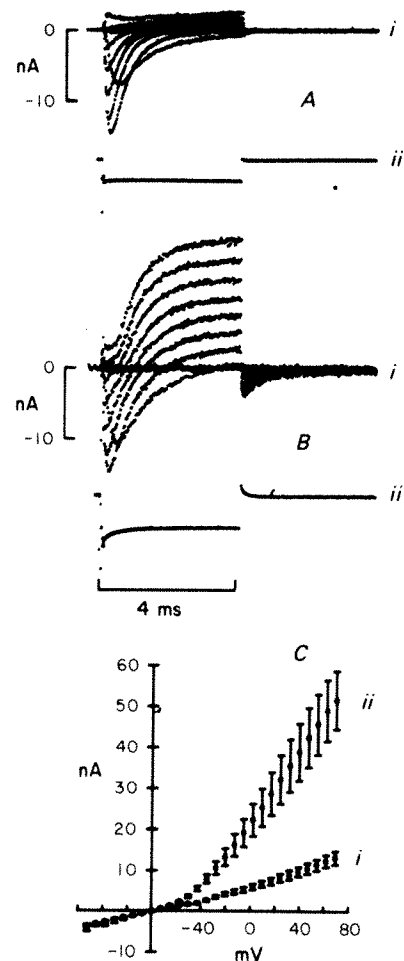


Fig. 1 Ionic currents in rabbit node before (Ai) and after (Bi) acute treatment to loosen the myelin. Families of current in both A and B were generated by the same series of depolarisations from an initial holding value of -80 mV to various test potentials in the range -72.5 mV to $+62.5$ mV in 15 mV increments. The currents were corrected for a linear leakage by appropriate scaling of the current response associated with a hyperpolarisation to -125 mV (Aii, Bii). The currents in A were measured in normal Locke after 30 min of repeated treatment (see text). During this period, the outward current and the nodal capacity showed practically no change. Currents in B were measured 3 min after A with the fibre in normal Locke when the late outward current suddenly showed a marked increase. Note that the capacity transient shows both a fast and a slow component; the latter is relatively small beforehand (Aii) whereas afterwards (Bii) it becomes much larger. The nodal capacity was obtained from records Aii and Bii by integrating numerically the capacity current during the pulse after correcting for a steady leakage. Since there were only a few computer sampling points during the fast capacity transient, the value for the capacity obtained by this procedure is based mainly on the slow component of current. The capacity per node obtained in this way was 2.5 pF in A and 35 pF in B. The steady leakage was 2.9 nA in A and 4.4 nA in B. Current signals were sampled every 30 μ s during the test pulse and every 150 μ s afterwards; and the time scale for the current after the pulse had been compressed fivefold compared to that used during the pulse. In addition, the fast capacity transients in A and B were sampled every 12 μ s. C shows the current-voltage relation of the total steady ionic current (mean values ± 1 standard error) for four fibres before (i) and after (ii) acute 'myelin-loosening' treatment (30–45 min). The steady currents were measured 4–8 ms after onset of the potential change. The holding potential was -80 mV for all experiments. The temperature was 24°C .

total capacity suddenly increased from an initial value of 2.5 pF to about 35 pF, with the increase coming mainly from the slow capacity current (see legend to Fig. 1). Interestingly, the dramatic increase in both the outward current and the capacity was not accompanied by a corresponding increase in the early sodium current (Fig. 1A,B), suggesting that the newly exposed axonal membrane has few sodium channels. Figure 1C shows the current-voltage relations of the total late current from four experiments in which both the increase in capacity and the outward current were sudden and particularly marked (the capacity increased by a factor of 20 ± 2.6 from an initial value of 3 ± 1.2 pF).

A simple explanation of these experiments is that the internodal axonal membrane, at least that in the paranodal region, normally contains potassium channels and the nodal axonal membrane does not. This idea is consistent with two recent experiments on mammalian nerve fibres in which the myelin was chronically disturbed by pathological means. First, Brismar³ has shown the consistent presence of potassium currents in alloxan diabetic rats; and it may be relevant that a characteristic of human diabetic neuropathy is a lifting of myelin away from the axonal membrane. Second, in rat dorsal root fibres exposed to diphtheria toxin, Sherratt, Bostock and Sears⁴ have shown that the outward membrane currents in regions of the nerve showing evidence of delayed saltatory or continuous conduction are sensitive to 4-aminopyridine and tetraethylammonium chloride, in contrast to their insensitivity at normal nodes of Ranvier as described by Bostock, Sherratt and Sears⁵. Finally, even in apparently normal nodes, potassium currents have occasionally been observed⁶, perhaps because the paranodal region was exposed by slight mechanical stretching during dissection.

The late outward current reported here is unlikely to be an artefact; and we believe that it is mainly a potassium current for the following reasons. First, it was blocked partially by external TEA and more effectively by allowing caesium and TEA to diffuse to the inside of the node. Second, the time constant of turn-on of this late current was voltage-dependent (as in Fig. 2D), and similar in size to the time constant for potassium

current observed previously in the frog. Thus as Fig. 2B shows the time course of the outward current in Fig. 2A can be reasonably well described by n^4 kinetics in much the same way as in frog fibres⁷. Finally, the outward current showed a reversal potential which became more positive when the external potassium concentration was increased. It seems, therefore, that the biochemical inhomogeneity previously described for sodium channels in mammalian nodal membrane by Ritchie and Rogart⁸ is accompanied by a corresponding complementary inhomogeneity in the distribution of potassium channels. Thus, virtually all the sodium channels are located in the nodal region whereas most of the potassium channels are located in the internodal region. The significance of this uneven distribution of potassium channels remains unclear.

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Tumour promoter uncouples β -adrenergic receptor from adenylyl cyclase in mouse epidermis

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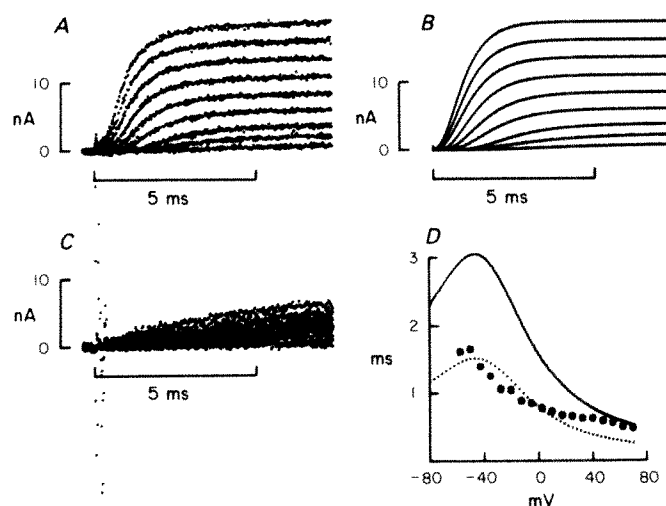


Fig. 2 Kinetics of the late outward current. A shows the effect of applying 100 nM tetrodotoxin on the family of currents in Fig. 1B: the early sodium currents were blocked and only the late outward currents were left. The currents in A were reconstructed in B using the expression $I = I_0[1 - \exp\{-(t - t_D)/\tau_n\}]^4$ with $I = 0$ for $t < t_D$. The voltage dependence of τ_n in the same experiment is shown in D (●). Also shown is the theoretical time constant (solid line) for the frog potassium current at 22 °C using the rate constants from ref. 6; for comparison with the rabbit data this curve has been scaled by a factor of 0.5 (broken line). C shows that the outward current is sensitive to internal Cs and TEA. The records were taken 10 min after changing the end pool solution in A from KCl to 80 mM CsCl and 80 mM TEA-Cl. Temperature, 24 °C.

Alterations in β -adrenergic receptor number and function and in the hormonal responsiveness of adenylyl cyclase have been observed in transformed cells¹⁻³, and tumours^{4,5}. Phorbol myristate acetate (PMA), a potent tumour promoter in mouse skin, induces a dramatic loss of epidermal responsiveness to catecholamines *in vivo*⁶⁻⁸, although basal levels of cyclic AMP are not affected⁷⁻⁹. In other work we have shown that PMA treatment does not alter the number or affinity of epidermal β -receptors, although accumulation of cyclic AMP in response to isoprenaline injection is sharply inhibited¹⁰. Evidence is presented here that PMA exerts this effect by uncoupling epidermal β -receptors from adenylyl cyclase.

Maguire *et al.*¹¹, and Lefkowitz and Williams¹² have established several criteria to distinguish well coupled β -adrenergic receptor-adenylyl cyclase systems from uncoupled or poorly coupled ones. Well coupled adrenergic systems from many cell types and tissues exhibit higher ratios of K_D (dissociation constant for agonist binding) to K_{act} (activation constant or concentration of agonist required to give 50% maximal response) than do poorly coupled systems. Agonist binding is decreased in coupled systems (K_D is increased) by the presence of guanine nucleotides, whereas guanine nucleotides do not affect agonist binding in uncoupled systems. Coupled systems often show lower K_D values and lower Hill coefficients for agonists than do uncoupled systems¹³.

We have measured cyclic AMP accumulation and β -receptor binding by the β -agonist (-)isoprenaline in epidermal homogenates from untreated and PMA-treated mice. Mouse epidermal β -adrenergic receptors have been previously identified and characterised in our laboratory¹⁰, using the labelled

β -antagonist ^3H -labelled (-)dihydroalprenolol (DHA)¹⁴. These receptors exhibit rapid and reversible equilibrium binding, saturability and stereospecificity for the L configuration of antagonists¹⁰.

Female 8-week-old Ha/ICR mice (Sprague-Dawley) were shaved, treated with 10 μg PMA (obtained from P. Borchert, Chemical Carcinogenesis, Eden Prairie, Minnesota) in 0.2 ml acetone or with 0.2 ml acetone alone and killed 12 h later. This interval was chosen because Grimm and Marks have shown the loss of epidermal β -adrenergic responsiveness to be maximal 12 h after PMA treatment⁶. Back skins were removed, stretched on cards epidermis side up, and frozen in liquid N_2 . Epidermis was removed by scraping as previously described⁸. Epidermal homogenates were prepared by homogenisation in a loose Dounce homogeniser (15 strokes by hand) in cold phosphate-buffered saline (PBS), pH 7.2. Agonist binding to homogenates was determined by competition with ^3H -labelled (-)dihydroalprenolol (NEN) as described in the legend to Fig. 3, and cyclic AMP was measured by radioimmunoassay⁸.

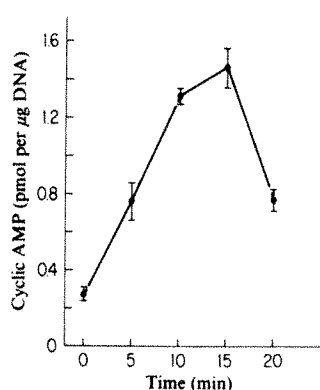


Fig. 1 Cyclic AMP accumulation in mouse epidermal homogenates treated with isoprenaline *in vitro*. Pooled epidermal homogenates were prepared from 15 skins as described in the text using 3 ml PBS per epidermis. In 25 ml flasks 2.4 ml of homogenate and 0.3 ml GTP (3 mM) were incubated at 37 °C for 15 min with shaking. Isoprenaline (30 μl) was added for a final concentration of 5×10^{-5} M and the flasks were incubated with shaking at 37 °C for the indicated times, at which point 0.2 ml 100% trichloroacetic acid was added. The flask contents were homogenised in a Polytron, the supernatant was extracted with ether and cyclic AMP was determined by radioimmunoassay as previously described⁸. DNA was determined by fluorometric assay²³. Values shown are means \pm s.e.m. of triplicate points.

Incubation of epidermal homogenates *in vitro* with isoprenaline results in an increase in cyclic AMP levels as shown in Fig. 1. The peak at 10–15 min, followed by a decline is characteristic of the *in vivo* epidermal response to intraperitoneal injection of isoprenaline^{6,7}. Figure 2 illustrates the dose-dependent effect of isoprenaline on homogenates from untreated and PMA-treated mice. The maximal stimulation of cyclic AMP accumulation in PMA-treated epidermis is significantly reduced from controls, and requires 10 times the concentration of isoprenaline. The K_{act} is increased approximately fourfold in the treated group.

Effects of GTP on isoprenaline binding to epidermal homogenates are shown in Fig. 3. The binding and activation data for control and PMA-treated epidermis, are summarised in Table 1.

From these data it seems that *in vivo* PMA treatment of mouse skin eliminates the effect of GTP on K_D and the pseudo-Hill coefficient (k_h) seen in control homogenates. In addition, both binding parameters are increased over controls without GTP. Of primary importance is the large drop (~sevenfold) in the K_D/K_{act} ratio after PMA treatment.

The concentration of isoprenaline required for 50% displacement of the labelled antagonist is considerably ($\sim 10^2$)

Table 1 Isoprenaline binding and activation parameters for untreated and PMA-treated mouse epidermis

	GTP* (300 μM)	Untreated	PMA-treated†
K_D (mM)	–	0.35	0.72
K_D (mM)	+	1.3	0.79
k_h ‡	–	–0.63	–0.85
k_h	+	–0.80	–0.86
K_{act} (mM)§	+	0.39	1.33
K_D/K_{act}	+	4.33	0.60

* GTP present (+) or absent (–) in the incubation mixture.

† PMA (10 μg in 0.2 ml acetone) applied percutaneously 12 h before death.

‡ Pseudo-Hill coefficient (slopes of plots in Fig. 3c, d).

§ K_{act} was determined only in the presence of GTP.

higher than that seen in other β -receptor systems. Not enough is known about the epidermal β -adrenergic system to properly evaluate this discrepancy, although in other work¹⁰ we found that the apparent K_D for antagonist (dihydroalprenolol) binding (52 nM) was also higher than other reported values^{11,12}. Comparably high levels of isoprenaline were required for stimulation of cyclic AMP accumulation as well as for binding. This may be a property of the *in vitro* epidermal preparation and incubation conditions used.

The agonist binding and activation results presented here provide evidence for a β -receptor-adenylate cyclase 'uncoupling' action by PMA. These results are consistent with data obtained using the uncoupling agent filipin^{15,16} and an uncoupled clone of S49 mouse lymphoma¹⁷. Several laboratories have found that incubation of cells with PMA results in a number of cell surface changes^{18–20}. Lee and Weinstein²¹ have shown that PMA interferes with binding of epidermal growth factor to HeLa cell membranes. An uncoupling mechanism for the loss of β -adrenergic responsiveness in mouse epidermis by PMA represents another facet in the action of phorbol ester on plasma membranes.

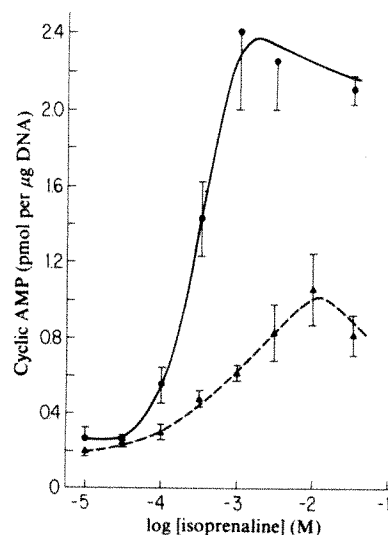


Fig. 2 Effect of PMA on isoprenaline-stimulated cyclic AMP accumulation in mouse epidermis *in vitro*. Epidermal homogenates were prepared from back skins of untreated (●) or PMA-treated (▲) mice killed 12 h after treatment. Incubation and cyclic AMP determination were carried out as described in the legend to Fig. 1, except that the concentration of isoprenaline was varied as indicated and the time of incubation was 12 min. Values shown are means \pm s.e.m. of triplicates.

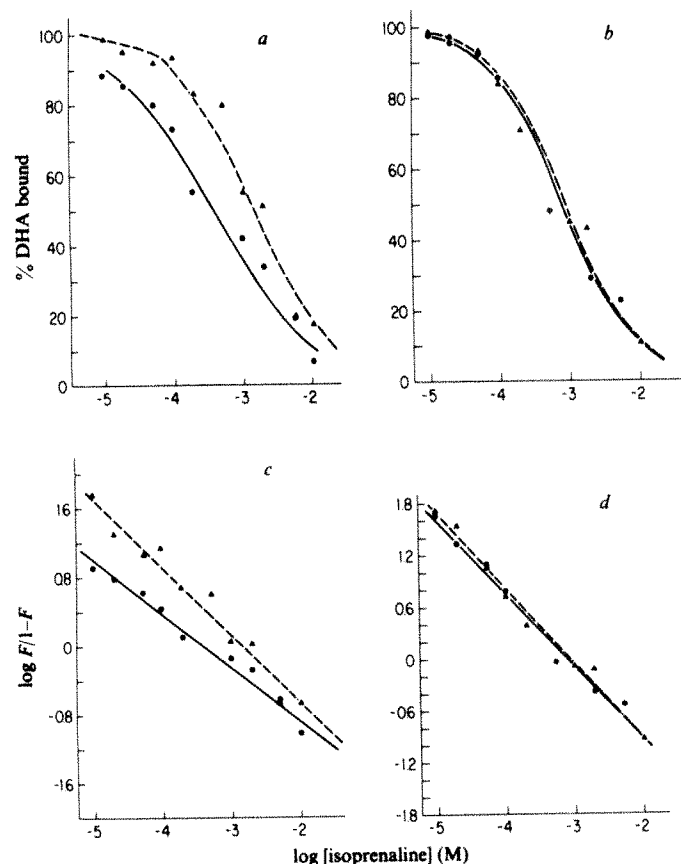


Fig. 3 Effects of GTP on binding of (-)isoprenaline to mouse epidermal β -receptors. Isoprenaline binding was measured by competitive displacement of 3 H-labelled DHA in the presence (▲) and absence (●) of 300 μ M GTP in untreated (a) and in PMA-treated (b) epidermis. Hill plots²⁴ for agonist binding in the presence (▲) and absence (●) of GTP for untreated (c) and PMA-treated (d) epidermis were calculated from the binding data. In triplicate tubes 0.2 ml of epidermal homogenate were incubated at 37 °C in a total volume of 0.5 ml containing PBS, 3 H-labelled DHA (5 pmol, 0.25 μ Ci) and (-)isoprenaline at indicated molar concentrations. GTP (0.15 μ mol) was included in some experiments; (-)propanolol (5 nmol) was added in duplicate tubes for determination of nonspecific binding. After 1 h, binding was terminated by addition of 2 ml ice-cold PBS and tube contents were immediately filtered through GF/D glass fibre filters (Whatman). The filters were thoroughly washed under vacuum with cold PBS, dried, and counted in 10 ml Aquasol (NEN). Counts from tubes containing (-)propanolol were subtracted from total counts to give specific binding. Non-specific binding of DHA represented between 30–50% of total counts. The concentration of DHA used (10 nM) gives 10% saturation of binding sites¹⁰.

Recent work on the β -adrenergic receptor–adenylate cyclase system suggests that the transduction of hormone binding into cyclase activation involves a ‘migration’ of receptors in a fluid membrane²². Phorbol ester, a lipid soluble membrane active compound, may interfere with the transduction or coupling process by altering the fluid or dynamic state of the membrane. The β -adrenergic uncoupling effect demonstrated here may be nonspecific and representative of a general loss or distortion of membrane function (in this case response to hormones) caused by PMA. The significance of the uncoupling effect for tumour promotion *in vivo* is indicated by a correlation between loss of adrenergic response and tumour promoting potency of a number of compounds⁶. An investigation of the coupling efficiency of epidermis undergoing two-stage chemical carcinogenesis and in skin papillomas and tumours is under way.

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Host genotype influences immunomodulation by interferon

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Interferon influences both afferent and efferent pathways of delayed-type hypersensitivity (DH) in the mouse. In animals previously sensitised to picryl chloride, sheep red blood cells (SRBC) or Newcastle disease virus (NDV), and treated with interferon just before challenge with any of these antigens, the antigen-elicited reaction, as measured by the ear-swelling (picryl chloride) or footpad swelling (SRBC and NDV) test, is either decreased or completely inhibited, depending on the dose of interferon administered^{1,2}. In addition to this action on expression of the sensitised state, interferon decreases or inhibits sensitisation to SRBC or NDV when administered 24 h before immunisation^{2,3}. These effects were recently confirmed using electrophoretically pure mouse interferon, thus ruling out the possibility that they are caused by other proteins present in the previously used partially purified interferon preparations⁴. For the effect on sensitisation, the timing of interferon administration is crucial, and when interferon is administered a few hours after the antigen, sensitisation can actually be enhanced, as reported here; the enhancement of sensitisation by interferon is influenced by the dose of antigen and by the genotype of the mice that are sensitised.

Sensitisation with sheep erythrocytes was achieved by the method of Lagrange *et al.*⁵. Briefly, mice are sensitised by an intravenous (i.v.) injection of 10^6 SRBC, followed 4 days later by challenge with 10^8 SRBC in 40 μ l of phosphate-buffered saline into the left hind footpad. Footpad swelling is measured 24, 48 and 72 h later with calipers and expressed as the difference in thickness between right (unchallenged) and left foot. Usually, 48-h values are 40–80% higher than 24-h values; at 72 h footpad swelling has significantly decreased.

The interferon used for these experiments was prepared in monolayer cultures of C-243 cells, induced with NDV after priming^{6,7}. It was then purified and concentrated either on blue Sepharose⁸ or on poly (U) Sepharose⁹, giving preparations with a specific activity of $10^{7.5}$ to 10^8 units per mg. For one experiment (Table 1) electrophoretically pure interferon, of specific

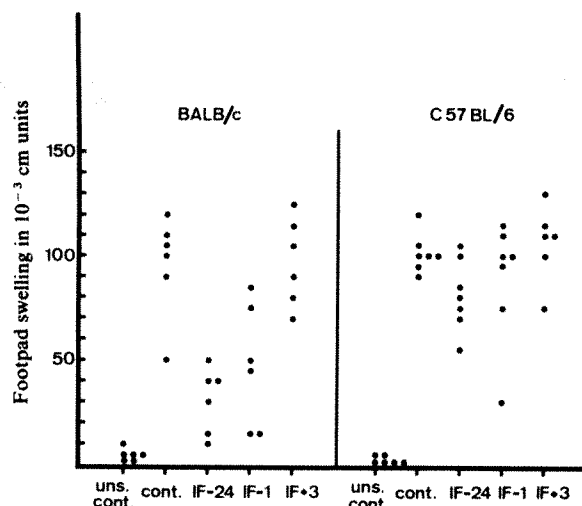


Fig. 1 Effect of timing of interferon administration on sensitisation. Three-month-old female BALB/c or C57BL/6 mice were sensitised with 10^6 SRBC. One group of each strain received an intraperitoneal (i.p.) inoculation of 3.3×10^5 interferon units in 1 ml either 24 h before (-24 h), 1 h before (-1 h) or 3 h after ($+3$ h) sensitisation. Footpads were challenged 4 days later; each point represents the 48-h reaction of one animal. See Table 2 for analysis of data.

activity 2×10^9 unit per mg, was used¹⁰. All interferon units are expressed in NIH reference units.

When interferon is given 24 h before immunisation with SRBC, inhibition of sensitisation is more pronounced in BALB/c mice than in C57BL/6 mice, and when it is given only 1 h before SRBC, inhibition of sensitisation no longer occurs in C57BL/6 mice whereas it is still significant in BALB/c mice. These results are summarised in Fig. 1 and Table 2. When interferon is administered 3 h after sensitisation, no significant difference in reaction after footpad challenge is observed between interferon-treated and controls either in BALB/c or C57BL/6 mice, provided they have been sensitised with an optimal dose of antigen (10^6 SRBC) (see also Fig. 1). However, if a sub-optimal dose of antigen is used (10^5 SRBC), a stimulating effect of the subsequent interferon treatment is observed, and this enhancement is more pronounced in C57BL/6 than BALB/c mice (Fig. 2a, Table 2). The difference in reaction to interferon between mice of the two strains is also evident with a dose of antigen that normally does not result in sensitisation. BALB/c mice sensitised with $10^{4.3}$ SRBC and challenged 4 days later do not develop significant footpad swelling, regardless of whether they have been treated with 3.3×10^5 units of interferon 3 h after immunisation. C57BL/6 mice, on the other hand, develop significant footpad swelling if they have been treated with interferon 3 h after immunisation with $10^{4.3}$ SRBC (Fig. 2b, Table 2). Thus, the immunosuppressive effect of exogenous interferon on the afferent pathway of DH to SRBC is more

pronounced in BALB/c than C57BL/6 mice, whereas the immunostimulating effect is more pronounced in C57BL/6 than BALB/c mice.

A striking difference between mice of the two genotypes also exists when the influence of interferon on expression of DH to SRBC is examined. When given a few hours before footpad challenge, interferon inhibits the expression of DH in both strains. However, it is less active in C57BL/6 mice. For example, a dose leading to a pronounced inhibition of footpad swelling in sensitised BALB/c mice remains without significant effect in C57BL/6 mice (Fig. 3, Table 2). About 25 times the amount of interferon used in BALB/c is needed to obtain comparable inhibition of footpad swelling in C57BL/6 (Table 1).

These results have several implications for the immunomodulatory effects of type I interferon. The timing of interferon administration with regard to the time of immunisation is crucial in determining whether inhibition or enhancement of sensitisation will occur. A similar observation was made in a study of the effects of interferon on antibody formation *in vitro*; when interferon is added before the antigen, inhibition of antibody formation is observed, whereas when interferon is added after the antigen, enhancement is sometimes obtained¹¹. In addition to timing, antigen dosage is important, and significant immunoenhancement only results when sub-optimal or normally non-sensitising doses of SRBC are used. In addition to stressing the importance of timing and antigen dose, our results show that immunomodulation by interferon is under the influence of host genotype, as the immunosuppressive effect of interferon on both afferent and efferent pathways of DH to SRBC is less pronounced in C57BL/6 than BALB/c mice, whereas, in contrast, the immunoenhancing effect is more readily obtained in C57BL/6 mice. These observations indicate

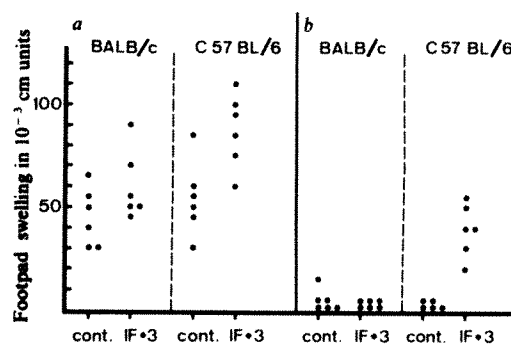


Fig. 2 Influence of antigen dosage and mouse strain on enhancement of sensitisation by interferon. Three-month-old female BALB/c or C57BL/6 mice were sensitised by an intravenous inoculation of either 10^5 (a) or $10^{4.3}$ (b) SRBC. The interferon-treated group received 3.3×10^5 units in 1 ml i.p. 3 h after sensitisation. The footpads were challenged 4 days later; each point represents the reaction of one animal, measured 48 h after challenge. See Table 2 for analysis of data.

Table 1 Effect of interferon on expression of DH to SRBC

BALB/c				C57BL/6			
	No. of mice	24-h footpad swelling in 10^{-3} cm units (mean \pm s.d.)	% Of untreated control		24-h footpad swelling in 10^{-3} cm units (mean \pm s.d.)	% Of untreated control	
Controls	6	52.5 \pm 16.0	—	6	65.0 \pm 13.7	—	
Interferon treated							
a	6	10.0 \pm 7.0	13 $P < 0.005$	6	40.0 \pm 23.1	63 $P < 0.025$	
b	6	28.3 \pm 10.3	54 $P < 0.01$	6	54.0 \pm 29.4	83 NS	
c	6	32.5 \pm 16.3	62 $P < 0.05$	6	56.0 \pm 6.0	87 NS	

The experimental protocol was essentially identical to that followed for the experiments summarised in Fig. 3, except that electrophoretically pure interferon (IF) was used. IF a: 2.2×10^5 units per ml; b: 4.4×10^4 units per ml; c: 8.8×10^3 units per ml. P , Level of significance of difference with control. NS, Not significant. Each animal received 1 ml of interferon.

Table 2 Statistical analysis of the data represented in Figs 1-3

Fig. 1		BALB/c		C57BL/6	
	Mean \pm s.d.	% Of control		Mean \pm sd	% Of control
Controls	95.8 \pm 24.5	—	—	101.4 \pm 9.4	—
IF-24 h	30.8 \pm 15.6	32	$P < 0.005$	81.4 \pm 17.2	80
IF-1 h	47.5 \pm 29.2	50	$P < 0.01$	89.2 \pm 29.0	88
IF+3 h	97.5 \pm 21.1	102	NS	106.6 \pm 18.3	105
					NS

Fig. 2		BALB/c		C57BL/6	
Sensitised with 10^5 SRBC					
		Control	Interferon		
				Mean \pm s.d.	
				40.0 \pm 23.0	—
				60.0 \pm 17.0	NS
				54.1 \pm 18.2	—
				87.5 \pm 18.1	$P < 0.005$

Fig. 3		BALB/c		C57BL/6	
Sensitised with $10^{4.3}$ SRBC					
		Control	Interferon		
				2.0 \pm 2.7	—
				35.8 \pm 19.6	$P < 0.005$

Fig. 3		BALB/c		C57BL/6	
		% Of control		% Of control	
24 h	Cont.	45.6 \pm 20.7	—	59.6 \pm 26.4	—
	IFa	11.1 \pm 8.7	24	40.4 \pm 24.9	68
	IFb	26.7 \pm 24.5	59	47.7 \pm 25.8	80
48 h	Cont.	82.5 \pm 24.0	—	93.2 \pm 33.3	—
	IFa	45.2 \pm 20.5	55	82.8 \pm 33.0	89
	IFb	54.1 \pm 31.6	66	89.5 \pm 26.0	96
					NS

P, Level of significance of difference with control. NS, Not significant.

that, in addition to genes influencing the production of interferon^{12,13}, there are genes in the mouse that influence the action of interferon on cells of the immune system; recombinant inbred and congenic lines derived from BALB/c and C57BL/6 mice will be instrumental for further genetic analysis of this phenomenon.

There are other examples of genes influencing interferon action. In the mouse, the *Mx* locus was recently shown to influence interferon action specifically and exclusively on orthomyxovirus replication (O. Haller, personal communication). In man, a gene (or genes) on chromosome 21 influences antiviral activity, and cells trisomic for this chromosome are more sensitive to the antiviral activity of interferon and also to its cell multiplication inhibitory activity¹⁴⁻¹⁶. Recently, a correspond-

ing gene has been localised in the mouse on chromosome 16 (L. Epstein, personal communication, and D. Slate, personal communication). This gene is probably not involved in the effects of genotype interferon action described here because when we compared mouse embryo fibroblast cultures from BALB/c and C57BL/6 origin for their sensitivity to the antiviral effect of interferon as measured by inhibition of VSV replication, no difference was found between the two genotypes (unpublished observations). Different sensitivities to the action of interferon therefore do not seem to be a general characteristic of all BALB/c and C57BL/6 cells. It is more likely that the difference is expressed at the more specialised level of T-cell, B-cell or macrophage function, and *in vitro* experiments comparing cells of both genotypes may clarify this point.

In addition to their theoretical interest, our results have a practical implication. Similar differences in immunomodulation by interferon according to genotype, if found in man, could explain the significant individual variations in the clinical effects of interferon that have been observed.

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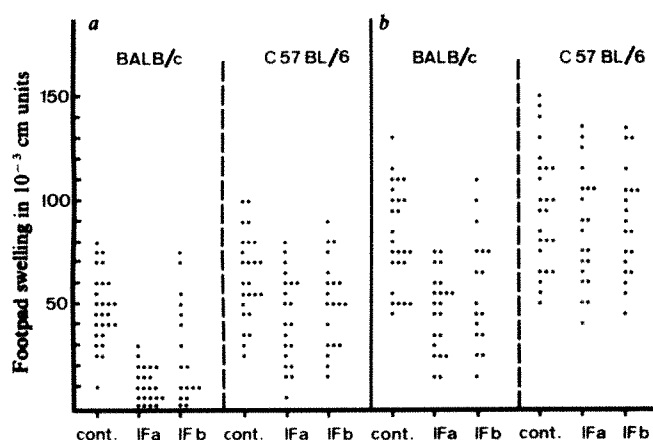


Fig. 3 Immunosuppressive effect of interferon on expression of DH to SRBC in BALB/c and C57BL/6 mice. The results of three different experiments are combined. Three-month-old-female BALB/c and C57BL/6 mice were sensitised by an i.v. inoculation of 10^6 SRBC. Four days later and 3 h before footpad challenge, the interferon-treated group received an i.p. inoculation of 1 ml containing 3.3×10^5 interferon units (IFa), 3.3×10^4 interferon units (IFb) or phosphate-buffered saline (cont.). Footpad swelling was measured 24 h (a) and 48 h (b) after challenge. Each point represents the footpad swelling of one mouse. The means \pm s.d. of the plotted values are given in Table 2.

Herpes simplex virus depresses antibody production by affecting T-cell function

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Many viruses enter lympho-reticular cells during pathogenesis and thereby induce immunosuppression¹, which is of practical importance in that it may be related to overall virulence². Immunosuppression may result from a selective infection, as viruses often show an affinity for different lymphocyte subpopulations: Epstein-Barr virus, for example, infects only a small percentage of B cells³. We reported previously that herpes simplex virus (HSV) type 1 suppressed the induction of an antibody response to diphtheria toxoid in cultures of human tonsil cells, and that this seemed to result from the infection of a small percentage of T lymphocytes⁴. However, as fully infectious virus was used in these experiments, it had probably spread from cell to cell in the course of the culture, so complicating the interpretation of the results. Accordingly, we have now re-investigated the mechanism of immunosuppression using temperature-sensitive (*ts*) mutants which fail to complete their growth cycle in the conditions selected for antibody synthesis. In this study, mutants *ts*B, *ts*D and *ts*F, derived from HSV type 1 strain 17, and *ts* 9, derived from HSV type 2 HG 52, were used. The results suggest that the immunosuppression is due to the selective infection by the viruses of helper T cells.

In four experiments a representative example of which is presented in Table 1, the HSV type 1 mutants *ts* B, *ts* D and *ts* F consistently suppressed the synthesis of both specific antibody and immunoglobulin as effectively as did wild-type HSV 1 and HSV 2. In contrast, the HSV type 2 mutant *ts* 9 had little effect. In these experiments, virus was added 24 h after antigen stimulation. Virus assays carried out in all experiments showed that the temperature-sensitive mutants grow in lymphocytes cultured at 31°C but not at the restrictive temperature of 37°C.

Susceptibility to the immunosuppressive effects of the virus was of limited duration, for *ts* F failed to affect antibody synthesis at 37°C when added 72 h or more after induction of the response. This is in keeping with our original observations⁴. After 7 d, the viability of cells in cultures infected with *ts* F, as measured by trypan blue exclusion, was 65% compared with 73% in uninfected cultures, indicating that immunosuppression did not result from an excessive death of lymphocytes.

Table 1 Immunosuppression by *ts* mutants of herpes simplex virus

Virus added	Diphtheria toxoid antibody (U ml ⁻¹)	IgM (μg ml ⁻¹)	IgG (μg ml ⁻¹)
Nil	2.13 ± 0.11	29.5 ± 0.7	57.0 ± 8.4
Type 1 <i>ts</i> ⁺	0.34 ± 0.02	9.8 ± 1.3	25.6 ± 2.1
<i>ts</i> B	0.36 ± 0.02	2.15 ± 0.8	26.5 ± 7.7
<i>ts</i> D	0.45 ± 0.03	9.5 ± 0.7	35.5 ± 3.5
<i>ts</i> F	0.39 ± 0.06	6.8 ± 2.1	37.0 ± 4.9
Type 2 <i>ts</i> ⁺	0.35 ± 0.03	1.9 ± 2.6	36.0 ± 5.6
<i>ts</i> 9	1.45 ± 0.37	24.4 ± 3.1	60.25 ± 3.1

Human tonsil lymphocytes were stimulated with diphtheria toxoid antigen and infected 24 h later with wild-type HSV or *ts* mutants at a multiplicity of infection (MOI) of 0.1 plaque-forming units (PFU) per cell. The cultures were maintained for 7 days at 37°C. The supernatants were assayed for specific antibody to diphtheria toxoid by double antibody radioimmunoassay and for Ig classes by solid phase radioimmunoassay⁴. Values are mean ± s.e.m. of duplicate assays on triplicate cultures from a single tonsil.

The temperature-sensitive block in the mutant *ts* F growth cycle occurs late, allowing the synthesis of structural proteins but not infectious progeny; infected cells could therefore be counted by indirect immunofluorescence in the absence of virus spread. As shown in Table 2, using *ts* F as inoculum, the proportion of infected cells in the population did not exceed 3.2%. Infectious centre assays carried out at 31°C on cultures infected at 37°C with *ts* D or *ts* F which induce immunosuppression and *ts* 9 which does not, showed that less than 1% of the cells supported virus growth. In further experiments, subpopulations of lymphocytes were infected selectively with *ts* F to determine whether the effect on B-lymphocyte activity was direct or indirect. This necessitated a departure from the standardised method described for immunoglobulin synthesis to a micro-culture method using pokeweed mitogen (PWM)⁵, because of the small numbers of cells available after separation. These experiments showed that only in cultures in which T lymphocytes had been infected was immunoglobulin synthesis impaired. In cultures in which uninfected T lymphocytes were cultivated with infected B lymphocytes, immunoglobulin synthesis was not significantly depressed (Table 3). Furthermore, the addition of uninfected T lymphocytes to cultures containing infected T and B lymphocytes, 2 h after mitogen stimulation, partially restored immunoglobulin synthesis (Table 4). Complete restoration was not achieved because the optimal cell concentration for synthesis was disturbed by these manipulations⁶.

Table 2 Per cent tonsil cells infected by *ts* mutants of HSV

Days post-infection		0	1	2	3	7
Mutant	Assay					
<i>ts</i> D	ICA	0.01	0.11	0.014	0.12	0.0
<i>ts</i> F	ICA	0.01	0.10	0.016	0.05	0.0
<i>ts</i> 9	ICA	0.01	0.02	0.013	0.01	0.0
<i>ts</i> F	IF	1.1	3.2	2.1	1.8	1.0

Infectious centre assays (ICA) were carried out on monolayers of baby hamster kidney (BHK) cells. Tonsil cells were infected initially with virus for 2 h at 37°C and cultured in the conditions described in Table 1 legend. Before assay the cells were washed three times in L15 medium, and incubated for 1 h at 37°C in medium containing anti-HSV antibody to neutralise extracellular virus, before washing a further three times in L15 at 4°C. The cells were resuspended at concentrations of 10–10⁶ cells ml⁻¹ in L15 medium containing 0.6% carboxymethylcellulose; 1-ml aliquots were added to BHK monolayers grown in 12-mm diameter, 24-welled plastic plates (Linbro, Flow Laboratories) which were then centrifuged at 300g for 10 min at 4°C. After 3 d incubation at 31°C the plates were fixed with 10% formal saline, stained with crystal violet and the number of plaques counted. Figures are mean of duplicate observations for the percentage of lymphocytes forming plaques. Immunofluorescence (IF) was carried out on cell samples collected daily from bulk cultures. The cells were washed twice in Dulbecco's phosphate-buffered saline, pH 7.4, transferred to Teflon-coated glass slides (Flow Laboratories), fixed in acetone for 15 min at -20°C and stored at -20°C. The preparations were stained with rabbit antisera to HSV type 1 which had been grown in rabbit kidney (RK13) cells, followed by fluorescein-conjugated goat anti-rabbit serum (Behringwerke) and counterstained with rhodamine-conjugated bovine serum albumin (Microbiological Associates). Preparations were mounted in buffered glycerol and examined under a Zeiss microscope (universal) fitted with a Ploem illumination system with separate excitation for the two fluorochromes. Cells were counted in non-overlapping fields until the total number in duplicate samples exceeded 1,000.

As expected, B lymphocytes cultured in isolation failed to synthesise immunoglobulin (Tables 3, 4), indicating that immunoglobulin synthesis in response to PWM is T-cell dependent⁶. The addition of 'T-helper factor' restored immunoglobulin synthesis by isolated infected or uninfected B lymphocytes to an equivalent extent (Table 4). These results confirm that HSV-induced immunosuppression results from a loss of T-helper function.

Table 3 Effect of *ts* mutants of herpes simplex virus on function of lymphocyte subpopulations

Combinations of lymphocyte subpopulations cultured	IgG ($\mu\text{g ml}^{-1}$)
Unseparated	61.75 ± 8.5
Unseparated (<i>ts</i> F)	<3.0
T+B	47.75 ± 4.1
T (<i>ts</i> F)+B	13.88 ± 5.0
T+B (<i>ts</i> F)	32.5 ± 8.48
T (<i>ts</i> F)+B (<i>ts</i> F)	<3.0
B alone	<3.0

Lymphocyte subpopulations were separated by the rosetting method of Gmelig-Meyling and Ballieux⁷. B-lymphocyte preparations obtained contained 98% immunoglobulin-bearing cells⁸, whereas T lymphocytes had $<1\%$. Cultured cells were stimulated with 0.1 ml pokeweed mitogen per 1×10^7 cells at 37°C for 16 h at a concentration of 1×10^6 cells ml^{-1} in Falcon flasks, in a growth medium of RPMI 1640 (bicarbonate buffered), 10% fetal calf serum and 100 U ml^{-1} each of penicillin, streptomycin and mycostatin. The cells were then washed and infected by exposure to *ts* F mutant of HSV at an MOI of 1.0 PFU per cell for 2 h at 37°C . Extraneous virus was removed by washing three times. Cells restimulated by the addition of 2 μl per well of pokeweed mitogen, prepared by standard methods, were cultured in growth medium in wells of microtitre plates at a concentration of 2×10^5 cells per well. The ratio of T lymphocytes to B lymphocytes in recombination experiments were the same as the ratio in the unseparated cultures, namely 1:2. The cells were cultured at 37°C in an atmosphere of 5% CO_2 in air for 7 days. Supernatants were collected and assayed for immunoglobulin by solid phase radioimmunoassay.

Table 4 T cells or T-cell factor restores immunoglobulin synthesis by B lymphocytes exposed to HSV

Combination of lymphocyte subpopulations cultures	IgG ($\mu\text{g ml}^{-1}$)
Expt 1	
T+B	40.0 ± 8.3
T(<i>ts</i> F)+B(<i>ts</i> F)	<3.0
T(<i>ts</i> F)+B(<i>ts</i> F)+T	16.25 ± 2.9
T(<i>ts</i> F)+B(<i>ts</i> F)+T(<i>ts</i> F)	5.0 ± 1.0
Expt 2	
B alone	0.33 ± 0.2
B lymphocytes + T-helper factor	7.4 ± 1.4
B lymphocytes + T-helper factor + HSV	6.6 ± 1.4
T-helper factor alone	<0.01

Expt 1: culture conditions were as described in Table 3 legend. In these cultures, 5×10^4 infected T lymphocytes were incubated with 1×10^5 infected B lymphocytes for 2 h after adding PWM. 5×10^4 of either uninfected or infected T lymphocytes were added. Supernatants were collected after 7 days and assayed for immunoglobulin by solid phase radioimmunoassay. **Expt 2:** 2×10^5 cells were cultured with PWM in microtitre plates for 7 days. B lymphocytes were cultured in a mixture of one part of a 24-h culture supernatant from PWM-stimulated, unseparated cells from the same tonsil and two parts fresh medium. HSV at an MOI of 0.1 was added to the infected cells on day 1 of culture.

Table 5 Failure of ultracentrifuged supernatants from HSV-immunosuppressed tonsil cultures to depress immunoglobulin synthesis in fresh cultures

Culture	IgG ($\mu\text{g ml}^{-1}$)
Immunised	15.5 ± 4.0
Immunised + immunosuppressed supernatant	18.2 ± 5.2
Immunised + control supernatant	22.0 ± 6.5

Supernatants from 7-day-old tonsil cultures immunosuppressed by HSV or from non-immunosuppressed controls were centrifuged at 25,000g for 1 h. New tonsil cells were cultured in the conditions described in Table 1 legend in a mixture of one part of supernatant and two parts of fresh medium. All cultures were assayed after 7 days for immunoglobulin production and virus infectivity. In addition, the original supernatants were tested for infectious virus particles. All virus assays were negative.

We have not detected significant amounts of interferon in cultures of tonsil cells infected with HSV⁴; furthermore, the medium in which infected cells have been cultured and in which free virus has been removed supports normal levels of antibody synthesis by fresh tonsil cells (Table 5).

Our findings indicate that HSV can suppress the antibody response of B lymphocytes to diphtheria toxoid by infecting only a small population of T cells and that this suppression does not depend on a complete cycle of virus replication. Beyond this the mechanism is unclear, for HSV type 1 mutants blocked early (*ts* B, *ts* D) or late (*ts* F) in infection at 37°C were equally immunosuppressive. In contrast, the early-block mutant of HSV type 2, *ts* 9, did not immunosuppress. Because *ts* 9 causes less disruption of host cell metabolism than the other mutants (H. Marsden, personal communication), it suggests that a very early event, such as depression of host cell protein synthesis, may be concerned in the mechanism of HSV-induced immunosuppression.

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Lymphocyte differentiation and major histocompatibility complex antigen expression in the embryonic thymus

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During embryogenesis, stem cells migrate from the bloodstream into the thymic rudiment where they proliferate and differentiate into T lymphocytes. The epithelial cells of the thymic stroma may influence these processes by providing hormonal and/or contact stimuli to the developing lymphoblasts^{1,2}. Recently, it has been shown that T cells 'learn' to recognise the major histocompatibility complex (MHC) antigens during thymic lymphopoiesis and become MHC-restricted. Their subsequent response to other antigens can only occur in the context of MHC antigens of the haplotype encountered in the thymus³. Little is known, however, of antigen expression on the thymic stroma which may provide the reference framework on which this MHC restriction is based. In this study we use monoclonal antibodies to show that antigens of the K and I regions of the MHC are detectable on cells of the embryonic mouse thymic stroma from around the 14th day of gestation, just when lymphocyte differentiation is commencing. Furthermore, I-region antigen (Ia antigen) expression is probably limited to thymic epithelium at this stage of gestation and we have not detected Ia on other epithelial tissues of the pharyngeal complex. This pattern of expression is consistent with a role for the thymic stroma in MHC restriction, perhaps by the selection of lymphoid cells for survival on the basis of their recognition of stromal MHC determinants.

Primary cultures of thymus and other tissues removed from CBA/ca (H-2^k), C57BL/10(H-2^b) and BALB/c (H-2^d) embryos were set up in multiwell plates (for details see legend to Table 1). Within 24 h of explantation, cultures were examined for expression of MHC and Thy-1 antigens by an indirect immunofluorescence assay using monoclonal antibodies followed by purified, class-specific, Fab preparations of goat antibodies to mouse IgG₂ or goat antibodies to mouse IgM conjugated with fluorescein or rhodamine (for details see legend to Fig. 1). In this way, the possibility of reactivity to antigens other than those under investigation is minimised and specificity is obtained as evidenced by the complete absence of labelling on control cultures exposed to conjugate alone or where tissues from inappropriate haplotypes are employed. The use of short-term cultures allows unfixed, viable cells to be labelled while avoiding changes in cell populations or cell phenotype that might occur in long-term cultures.

Table 1 MHC and THY-1 antigen expression on embryonic mouse thymus and other organ rudiments

Expt	Age at explantation (d)	Material	Labelling		
			H-2K	H-2I	Thy-1.2
1	13	CBA thymus culture	—	—	NT
		C57BL/10 thymus culture	—	—	NT
2	13	CBA thymus culture	—	—	NT
3	14	CBA thymus culture	NT	+	NT
		BALB/c thymus culture	NT	—	NT
4	14	CBA thymus culture	+	+	NT
		BALB/c thymus culture	—	—	NT
5	14	CBA thymocyte suspension	NT	—	NT
6	14	CBA thymus culture	+	+	—*
		CBA salivary gland culture	+	—	—
7	15	CBA thymus culture	+	+	—*
		CBA head and neck skin culture	+	—	NT
8	15	CBA thymocyte suspension	+	NT	+
9	15	CBA thymus culture	+	+	NT
		CBA lung rudiment culture	+	—	NT
		CBA thyroid culture	+	—	NT

Embryonic thymus and other organ rudiments were dissected from mouse embryos at 13–15 days of gestation (day of plug = day 0), torn open with watchmakers forceps, washed and incubated at 37 °C for 30 min in 0.25% trypsin. Trypsinisation was terminated by centrifugation through a large volume of RPMI 1640 containing 10% fetal calf serum (RF-10) and the products of 10–15 organ rudiments resuspended in 200 µl of the same medium. Aliquots of cell suspension (40 µl) were placed in the wells of Sterilin immunofluorescence trays and cultured overnight. Suspensions of embryonic thymocytes were obtained just before assay by teasing organ rudiments in a small pool of medium. NT, not tested; +, labelled; —, not labelled.

* Double labelling experiments indicated the presence of some Thy-1.2-positive, Ia-negative cells; see text.

The results summarised in Table 1 indicate that K and I-region products of the MHC cannot be detected on cultured stromal elements of day 13 embryonic mouse thymus, but they are readily demonstrable by day 14 of gestation. The majority of labelled cells are located in cell aggregates and in the outgrowths derived from them (Fig. 1). These aggregates represent portions of thymus incompletely dispersed by the disaggregation procedure. We have examined these cell aggregates by transmission electron microscopy and find that they contain many epithelial cells characterised by tonofibrils and intercellular junctions. In

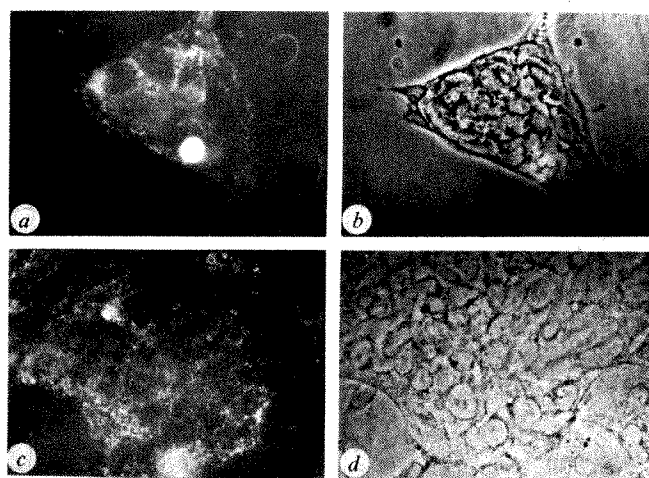


Fig. 1 Immunofluorescence (a) and phase contrast (b) micrographs of an island of epithelial-like cells in embryonic mouse thymus culture treated with monoclonal anti-Ia antibody. Note punctate fluorescent labelling on cell membranes. c and d, Immunofluorescence and phase contrast micrographs of a similar thymus culture labelled with monoclonal anti-H-2K. Antisera, monoclonal (IgG₂) anti-Ia. 17 (I^k) and monoclonal (IgG₂) anti-H-2K^k (both culture supernatants) used at a dilution of 1 in 4 in RF-20 and monoclonal (IgM) anti-Thy-1.2 (F7D5 ascitic fluid) used at a dilution of 1 in 20 were a generous gift from Drs Beverley and Lake, University College, London. Details of the anti-MHC antisera, which originated from the Department of Genetics, Stanford, USA, coded 10-3.6 and 11-4.1 respectively are given in ref. 9. Fluorescein- and rhodamine-conjugated purified class-specific antibodies to IgG₂ and IgM were a generous gift from Drs Cooper and Lawton, Birmingham, USA and have been described in detail elsewhere¹⁰. Before use on embryonic tissues, monoclonal reagents were checked for specificity and reactivity on adult mouse spleen cells (anti-MHC reagents) and on adult mouse and rat thymocytes (anti-Thy-1.2) (data not shown). Labelling of monolayer cultures were carried out by aspiration of medium from culture wells, addition of 20 µl of antiserum dilution and incubation at 37 °C for 30 min. Wells were washed individually by addition and aspiration of 50 µl of RF-20 followed by addition of 10 µl of rhodamine-conjugated, class-specific Fab preparations of goat anti-mouse IgG₂, fluorescein-conjugated goat anti-mouse IgG₂, or rhodamine-conjugated goat anti-mouse IgM (all at 0.5 mg ml⁻¹) as appropriate. Following 30 min incubation at room temperature, individual wells were washed five times with RF-20, fixed with methanol and a small circular cover slip (Chance no. 0, 6 mm diameter) mounted on 30% glycerol in phosphate-buffered saline was dropped into each well. For simultaneous labelling of Ia and Thy-1 antigens, wells were incubated first with a mixture of anti-Ia and anti-Thy-1 antisera followed by rhodamine-conjugated anti-IgM and fluorescein-conjugated anti-IgG₂. All cultures were examined using a Zeiss Universal microscope with epi-illuminator III/RS, Lamp HB0 50 and standard filter sets 9 or 15.

preliminary studies, using ferritin-conjugated anti-mouse Ig antibody to detect monoclonal anti-Ia antibody binding in the electron microscope, we also find that ferritin labelling of epithelial cells can be demonstrated in the appropriate (H-2^k) strain. In view of these results, it seems likely that many, although not necessarily all, of the aggregate cells expressing K and I-region products from the 14th day of gestation onwards are epithelial cells.

Thy-1-positive cells are detectable within and outside cell aggregates, but in double labelling experiments using anti-IgG₂-fluorescein to detect binding of anti-Ia antibody and anti-IgM-rhodamine to detect binding of anti-Thy-1 antibody, Ia- and Thy-1-positive cells are found to be distinct entities. Suspensions of lymphoid cells prepared by 'teasing' 14-day embryo thymuses are Thy-1 positive, but Ia-negative, when treated with the same reagents. It seems likely, therefore, that most, if not all of the Thy-1-positive cells detected within and outside cell

aggregates are lymphoid cells. Although we cannot exclude the possibility that some thymic stromal cells are Thy-1-positive, as suggested in a previous study using alloantisera on adult thymus cultures⁴, our results do indicate that Ia-positive, embryonic thymic epithelial cells are Thy-1-negative. In this context, it may be noted that the stromal cells of our cultures are heterogeneous since, outside aggregates, long spindle-shaped cells are Ia-negative.

In contrast to cultures of thymus, which express antigens of both K and I regions, epithelial tissues derived from salivary gland, head and neck skin, thyroid and lung rudiments, while expressing K region antigen, do not express Ia. This selective expression of Ia, in the site of T-cell differentiation, strengthens the argument that MHC antigens on thymic epithelial cells play a role in the development of restricted antigen recognition by T cells. In particular, since MHC restriction of the various T-cell subsets involves I as well as K and D-region antigens^{3,5,6}, it would be necessary that thymic epithelial cells express Ia antigens as well as the more ubiquitous K and D-region products from the commencement of lymphopoiesis. The present findings provide direct evidence that this is the case. Further analysis, however, is required to clarify the MHC status of non-epithelial stromal cells which could also contribute to this process. In accordance with our findings in the mouse embryo, the use of heterologous antisera on frozen sections has indicated the presence of Ia-like antigens in the adult guinea pig thymus⁷. Similarly, the expression of K and I region-products on thymic epithelial cells of the postnatal mouse has now been reported⁸.

The precise means whereby interactions between lymphocytes and embryonic thymus stromal cells take place is unclear. In view of the absence of Ia on the differentiating T-lymphoid cells and the corresponding absence of Thy-1 on the thymic epithelial cells, it would seem unlikely that interaction is facilitated by the mutual expression of these surface components on lymphoid and epithelial elements respectively. It is also unlikely that Ia expression on thymic epithelial cells is involved in the process of stem cell migration to the thymus, since migration is initiated some days before we can detect Ia expression. A possible role for epithelial Ia antigen in the initiation of stem cell proliferation following their entry into thymic rudiment is, however, conceivable. Nonetheless, the major function of MHC expression on thymic epithelial cells probably relates to the acquisition of MHC restriction by T cells.

Thus our studies show that K and I-region products of the MHC can be detected on cultures of thymic stromal cells from as early as the 14th day of gestation. While the first lymphoid stem cells probably enter the thymic rudiment from about the 11th day of gestation, the major proliferative burst is occurring from about the 14th day onwards. In the context of MHC restriction, it may be significant that antigens of the MHC regions involved in this process become apparent on the thymic epithelium at this time.

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V-J joining of immunoglobulin κ genes only occurs on one homologous chromosome

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In general, heterozygous animal cells express both alleles at a particular locus. The only exceptions are cells of XX genotype after inactivation of one X chromosome¹, and immunoglobulin-producing cells; in each case only one of the two alleles is expressed in differentiated cells and their progeny². This phenomenon, termed allelic exclusion, has been described for several mammalian species including man and mouse. It has been shown that the variable (V) and constant (C) region genes of immunoglobulins undergo a rearrangement during ontogeny^{3,4}. We wished to test whether allelic exclusion in B cells could be the consequence of V- and C-region rearrangement on one of the two homologous chromosomes only. For that reason we chose to analyse the rearrangement of immunoglobulin light chain genes in normal B lymphocytes isolated on the fluorescence-activated cell sorter. We now present evidence that during normal B-lymphocyte differentiation V-C rearrangement occurs only on one chromosome.

In germ-line (sperm) and embryo DNA the variable and constant region genes coding for immunoglobulin heavy and/or light chains are on widely separated DNA segments, whereas in neoplastic plasma cell clones (myelomas) V- and C-region genes are found closer together. Thus, at some point during differentiation of plasma cells (or their B-cell precursors) V- and C-regions are rearranged to form a functional transcription unit. Allelic exclusion could therefore be the consequence of V- and C-region rearrangement on only one of the two chromosomes. The homologous chromosome would not undergo any change and therefore remain silent with respect to expression of the allelic immunoglobulin genes. Restriction fragment analysis of myeloma DNA has led to contradictory results concerning V-C rearrangements on homologous chromosomes: in some myelomas V-C rearrangement seems to have taken place on one chromosome only, as both new V-C gene arrangements and V- and C-containing DNA restriction fragments of the same size as in embryo DNA were found⁵. In other myelomas, only the newly arising restriction fragments carrying the rearranged V and C genes could be detected^{3,4} (R.J., unpublished observation for MOPC 167). This latter finding indicated that both homologous chromosomes might be involved in the translocation process that leads to a functional immunoglobulin gene. As most myelomas are aneuploid tumours carrying several chromosomal aberrations, it is not possible to use them as models for the study of the phenomenon of allelic exclusion.

Figure 1 shows a restriction map of the C_{κ} gene in germ-line and embryo DNA⁶. The C_{κ} gene is close to one end on a

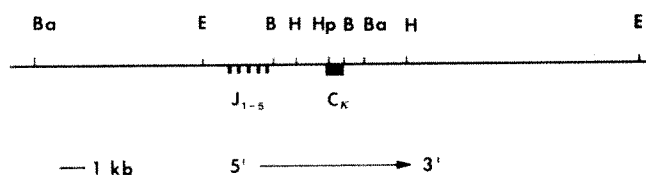


Fig. 1 Restriction map of the C_{κ} gene in germ-line and embryo DNA⁶. The position of the J regions J₁₋₅ is according to Max *et al.*⁷ and Sakano *et al.*⁸. The arrow indicates the direction of transcription. The following restriction enzymes were used: *Bam*HI (Ba); *Bgl*II (B); *Eco*RI (E); *Hind*III (H); *Hpa*I (Hp). kb, kilobase.

13-kilobase *Bam*HI fragment. Five J-region segments had been identified about 2.5–4.0 kilobases from the 5' end of the C_κ gene^{7,8}. Analysis of κ -chain clones from myeloma DNA had revealed that the 3' end of the V_κ to be expressed (including its 5'-flanking sequence⁹) was joined to the 5' end of a J region^{5,8}. Comparative analysis of restriction fragments of embryo and myeloma DNA resulted in restriction fragments of different sizes. The restriction enzyme *Bam*HI seemed to be a good choice for allowing V–J joining, because this event should always alter the 13-kilobase *Bam*HI fragment and create a V_κ - and C_κ -bearing *Bam*HI fragment of a different size. In fact, all κ -producing myelomas so analysed show new *Bam*HI fragments containing V_κ and C_κ sequences^{3–5}. Each new restriction fragment created by V–C translocation could differ in size from one lymphocyte to the other depending on which variable region has joined with which J region. As there are a large number of germ-line V_κ genes, *Bam*HI restriction fragment analysis of κ -light chain-producing cells does not necessarily show any particular new fragments, because the specific rearrangement of each lymphocyte would not be detected in a heterogeneous population of κ -bearing B cells. If V–C region rearrangement involved both homologous chromosomes, a disappearance of the 13-kilobase *Bam*HI germ-line fragment should be seen. However, if this event occurs on one chromosome only, the 13-kilobase *Bam*HI fragment should be retained.

We isolated splenic B lymphocytes having surface κ light chain. Staining of spleen cells with rabbit anti- κ immunoglobulin (Ig) and fluorescent goat anti-rabbit Ig antibodies yielded two different populations of cells. About 50–55% of spleen cells gave positive fluorescence when stained with a

rabbit anti- κ Ig whereas the remaining 45–50% did not stain above background (controls for background were the same cells stained at the same concentration with rabbit anti-keyhole limpet haemocyanin antibodies and the fluorescent goat anti-rabbit Ig). These two populations were purified on the fluorescence-activated cell sorter¹⁰. We isolated κ -positive (κ^+) from κ -negative (κ^-) cells. To minimise contamination, cells weakly positive for κ were not sorted as κ^+ cells. Contamination of sorted κ^+ with κ^- cells was less than 4%. DNA of κ^+ cells were purified, digested with *Bam*HI and electrophoresed through agarose to separate the resulting restriction fragments. After transfer of the DNA to DBM paper¹¹, restriction fragments bearing κ -region genes were detected by hybridisation with a nick-translated plasmid containing a purified C_κ -region insert⁶.

Table 1 Relative band intensities of various DNA digests

DNA digest	Relative band intensity	
	C_κ	Ribosomal
5 μ g <i>Bam</i> HI sperm	85	245
10 μ g <i>Bam</i> HI sperm	172	561
10 μ g <i>Bam</i> HI κ^+	87	664

The relative band intensities were determined by densitometry. Analysis of *Bam*HI-digested DNA with CD18 was used as internal standard and the intensities of all bands per lane were added up.

Figure 2 shows the results of this experiment. DNA from κ^+ cells is compared with different amounts of sperm DNA. The 13-kilobase *Bam*HI germ-line fragment is clearly detectable in κ^+ DNA. We interpret this to demonstrate that both homologous chromosomes have not undergone a rearrangement with respect to their V_κ and C_κ genes. If V–J joining takes place on one chromosome one would expect a 13-kilobase *Bam*HI fragment of half the intensity compared with the same amount of germ-line (sperm) DNA. To control for possible inaccuracies in the determination of the input DNA amounts, the band intensities were compared with an internal standard. For this, the DBM paper was washed in 0.4 M NaOH for 30 min, neutralised and rehybridised to a ³²P-labelled plasmid containing a ribosomal DNA insert¹². These results are shown in Fig. 2 and summarised in Table 1. The intensity of the ribosomal bands of 10 μ g sperm and 10 μ g κ^+ DNA after *Bam*HI restriction is the same. However, the intensity of the 13-kilobase C_κ fragment of 10 μ g κ^+ DNA corresponds to that of the 5 μ g sperm DNA. This is in agreement with the idea that V–C joining occurs on one chromosome during differentiation of κ -chain-expressing B lymphocytes. These experiments clearly demonstrate that in the majority of B cells V–J joining and therefore the formation of a functional transcription unit for κ chains does not occur on both chromosomes. However, a small fraction of B cells could have undergone V–J joining on both chromosomes and would not have been detected by this assay. We speculate that the consequence of V–J joining on one chromosome is the allelic exclusion of immunoglobulin gene products in B cells and plasma cells. Given that allelic exclusion may result from differential V–J joining, one must consider potential molecular mechanisms for this event. A reasonable assumption is that differentiating lymphoid cells of the B-cell lineage contain some highly specific enzymes that accomplish the V–J joining. It is not clear how these putative recombinases (or other enzymes effecting V–J joining) use only one of two identical chromosomes as their substrate and leave the second (homologous) one unchanged. Several possible mechanisms could lead to the activation of immunoglobulin genes on one chromosome only. One can imagine the production of one essential enzyme molecule (either the recombinase or a molecule that renders the chromosome susceptible to recombinase action) per cell that works in a stoichiometric fashion—one molecule per chromosome. Once V–J joining has been initiated the enzyme molecule is no longer active. Differentiating lymphoid cells that do not produce this enzyme would not enter the B-cell lineage.

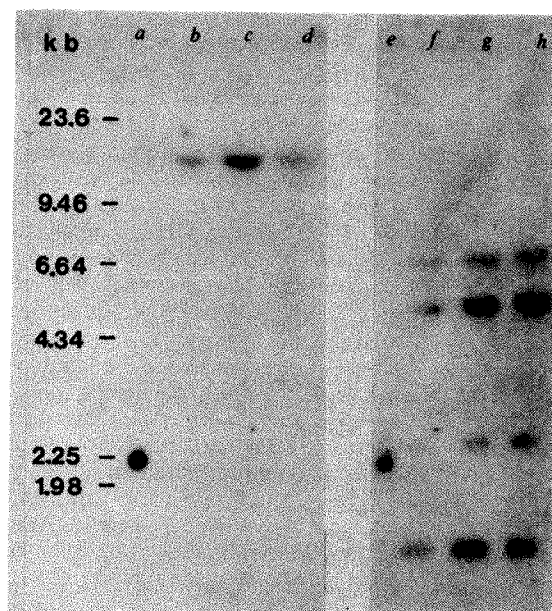


Fig. 2 A spleen cell suspension from BALB/c mice was treated on ice with 10 vol 8.3 g l⁻¹ NH₄Cl and 1 g l⁻¹ KHCO₃, pH 7.4, to lyse erythrocytes. The nucleated cells were collected by centrifugation, washed, stained with 40 μ g ml⁻¹ rabbit anti-mouse κ antibodies and counterstained with 75 μ g ml⁻¹ fluorescent goat anti-rabbit Ig antibodies. About 55% of the cells stained with the anti- κ antibodies. The brighter 50% of the spleen cells were sorted on a fluorescence-activated cell sorter⁹ (FACS III, Becton Dickinson). On re-analysis, this population was about 96% κ^+ . DNA was purified and analysed with *Bam*HI as described elsewhere⁶. The restriction fragments were electrophoresed through 0.7% agarose and transferred to DBM-paper¹⁰. Hybridisation was to ³²P-labelled C_κ probe (lanes a–d) or a plasmid containing a *Xenopus laevis* ribosomal DNA insert (CD18)¹¹ (lanes e–h). a, e, 1 μ g sperm DNA; b, f, 5 μ g sperm DNA; c, g, 10 μ g sperm DNA; d, h, 10 μ g κ^+ DNA. The molecular weight standard is *Hind*III-digested λ DNA.

A slightly different possibility is that a limiting amount of the enzyme is produced. If the enzyme level is too low no V-J joining occurs, and these cells will not become B lymphocytes. At higher enzyme levels one chromosome can be activated, leading to immunoglobulin-producing cells. At still higher levels (or infrequently at low enzyme levels) one would expect a rearrangement of V and C genes on both chromosomes in a small subpopulation of differentiating cells. This could lead to death of those cells if the V-C interval contains gene(s) essential to cell viability, and if this interval is excised during differentiation. A third possibility would be that as soon as rearrangement has been initiated a negative feedback prevents new rearrangements from taking place. Whatever the situation, selective chromosomal expression in the case of the κ chain accompanies selective chromosomal rearrangements. In

contrast, X-chromosomal allelic exclusion is apparently the consequence of selective chromosomal inactivation¹³. Nevertheless, it is possible that both activation and inactivation of chromosomal segments may result from selective irreversible chromosomal rearrangements.

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Evidence for the involvement of H1 histone phosphorylation in chromosome condensation

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Changes in the structural organisation of chromatin are necessary for the progression of the cell cycle. These changes are thought to be regulated mainly by the modification of chromosomal proteins by reactions such as phosphorylation¹⁻⁸, acetylation⁹⁻¹², methylation^{13,14} and poly(ADP-ribosylation)^{15,16}. Among these modifications, phosphorylation of histones, especially that of the H1 histone, is the most likely candidate for a factor which regulates chromosome condensation¹⁻⁸. It has been proposed that the phosphorylated form of H1 histone may be involved in the initiation of chromosome condensation¹⁻⁴ or in the maintenance of the condensed state of chromatin⁸. The latter possibility is unlikely because zinc chloride, which inhibits phosphatase *in vivo* and preserves the highly phosphorylated form of H1 histone at metaphase, does not prevent the dispersion of chromosomes at the end of mitosis¹⁷. As for the former possibility, Bradbury *et al.* reported² that the activity of H1 histone phosphorylation is closely correlated with mitotic triggering in the cell cycle and suggest^{3,4} that H1 histone phosphokinase is involved in the initiation of mitosis. However, no causal relationship has been established. To analyse the mechanism of the progression of the cell cycle, we have isolated several temperature-sensitive (ts) growth mutants from FM3A, a cell line derived from C3H mouse mammary carcinoma¹⁸. We report here on one of these ts mutants, designated the ts85 strain, which shows an abnormal chromosome condensation as well as deficiency in H1 histone phosphorylation at the non-permissive temperature. Our results support the hypothesis that H1 histone phosphorylation controls the initiation step of mitosis through chromosome condensation.

The ts85 cells were obtained as follows. Logarithmically growing FM3A cells in suspension culture were treated with 0.3 $\mu\text{g ml}^{-1}$ N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) for 16 h at 33 °C and incubated for 3 days of expression time. Wild FM3A cells were killed by the addition of ³H-thymidine (15 Ci mmol⁻¹ 5 $\mu\text{Ci ml}^{-1}$) for 16 h after the cells had been shifted up to 39 °C, the non-permissive temperature for ts cells. The selection schedule was repeated several times and then the surviving cells were inoculated into soft agar plates^{19,20} and incubated at 33 °C (permissive temperature) for about 2 weeks. The colonies which developed were checked for growth at 33 °C and 39 °C. The ts85 was one of the clones which showed no increase in the cell number at 39 °C but normal growth at 33 °C.

The ts85 cells were synchronised at the G1/S boundary by double treatment with excess thymidine at 33 °C and then permitted to enter the S phase at 33 °C or 39 °C. The amount of DNA synthesised at 39 °C was almost the same as that at 33 °C, but no mitotic cells were detected at 39 °C (Fig. 1), indicating that at the non-permissive temperature ts85 cells were arrested before the mitotic phase. When ts85 cells growing logarithmically at 33 °C were shifted up to 39 °C and incubated for 16 h, using a cytofluorometer, most of the cells were found to be arrested at the G2 phase. Details of the growth characteristics of ts85 will be reported elsewhere²¹.

To determine whether or not ts85 cells contain condensed chromatin at the arrested state at 39 °C, electron microscopic observations were carried out. As shown in Fig. 2, a small fraction of the chromatin was condensed into a fragmented form (arrow in Fig. 2), but the structures of the nuclear membrane and nucleoli were preserved. The incomplete condensation was observed in 80% of the total population after incubation for 16 h at 39 °C. Thus, most of the chromatin did not initiate condensation at the arrested state and as a result, the cells were not able to enter the mitotic phase.

If H1 histone phosphorylation stimulates the initiation of chromosome condensation¹⁻⁸, cells with a defect in chromosome condensation might have a deficiency in H1 histone phosphorylation. This is the case with ts85 cells. These cells were synchronised at the G1/S boundary, released from the blockade, incubated at 33 °C or 39 °C and the phosphorylation pattern of the histones was examined. Figure 3 shows the incorporation of ³²P and the densitometric profile of histones isolated from G2, M phase cells and cells arrested at 39 °C. The rate of phosphorylation at 39 °C of the H1 histone decreased to 1/10th of that at 33 °C (Fig. 3). The rates of phosphorylation of H2A and H4 histones, however, were somewhat higher at 39 °C

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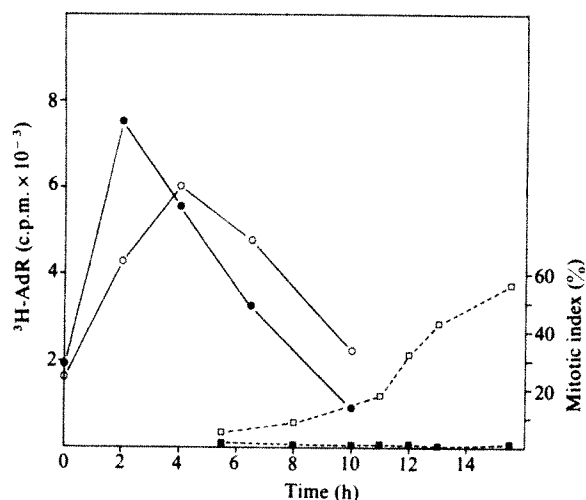


Fig. 1 DNA synthesis and entry into mitosis after synchronisation at the G1/S boundary. Logarithmically growing ts85 cells in suspension were treated with excess thymidine (2 mM) in RPMI-1640 containing 10% dialysed calf serum at 33 °C for 18 h. After removing excess thymidine, cells were incubated at 33 °C for 8 h in normal medium, then the second treatment with excess thymidine for 10 h at 33 °C was given. The ts85 cells which accumulated at the G1/S boundary were permitted to enter the S phase at 33 °C or 39 °C in normal medium containing 0.03 $\mu\text{g ml}^{-1}$ colcemid (Ciba). DNA synthetic activity at 33 °C (○) or 39 °C (●) was measured by pulse labelling with ^3H -deoxyadenosine (AdR, 1 $\mu\text{Ci ml}^{-1}$, 21 Ci mmol $^{-1}$) for 30 min at each temperature. The alkaline-resistant radioactivity in acid-insoluble materials collected on Whatman glass fibre filters (type GF/C) was measured by liquid scintillation spectrometer. Mitotic index at 33 °C (□) or 39 °C (■) was measured by microscopic observation of cells treated with 1% sodium citrate and fixed with methanol/acetic acid (3:1 v/v).

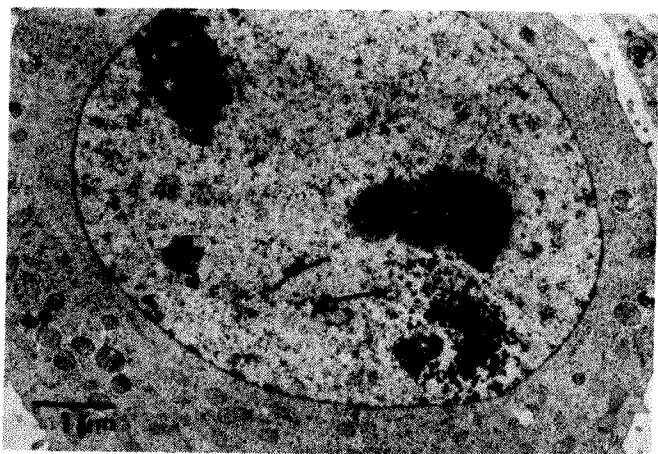


Fig. 2 Electron microscopic observation of a ts85 cell possessing fragmented abnormal condensation of chromatin (indicated by arrow) in the nucleus at 39 °C. Logarithmically growing ts85 cells at 33 °C were incubated at 39 °C for 16 h. After incubation, the cells were washed with 0.1 M phosphate buffer (pH 7.4) and fixed with phosphate-buffered 2.5% glutaraldehyde (pH 7.4) for 1 h at room temperature. After a wash with the same buffer, cells were post-fixed with 1% osmium tetroxide in the same buffer for 1 h at room temperature. The fixed cells were embedded in Epon-Araldite, cut into thin sections using a Sorvall ultramicrotome and observed with a JEOL 100B transmission electron microscope operated at 80 kV. In wild-type cells at both temperatures and ts85 cells at 33 °C, no such fragmented abnormal condensation of chromatin was detected throughout their cell cycles.

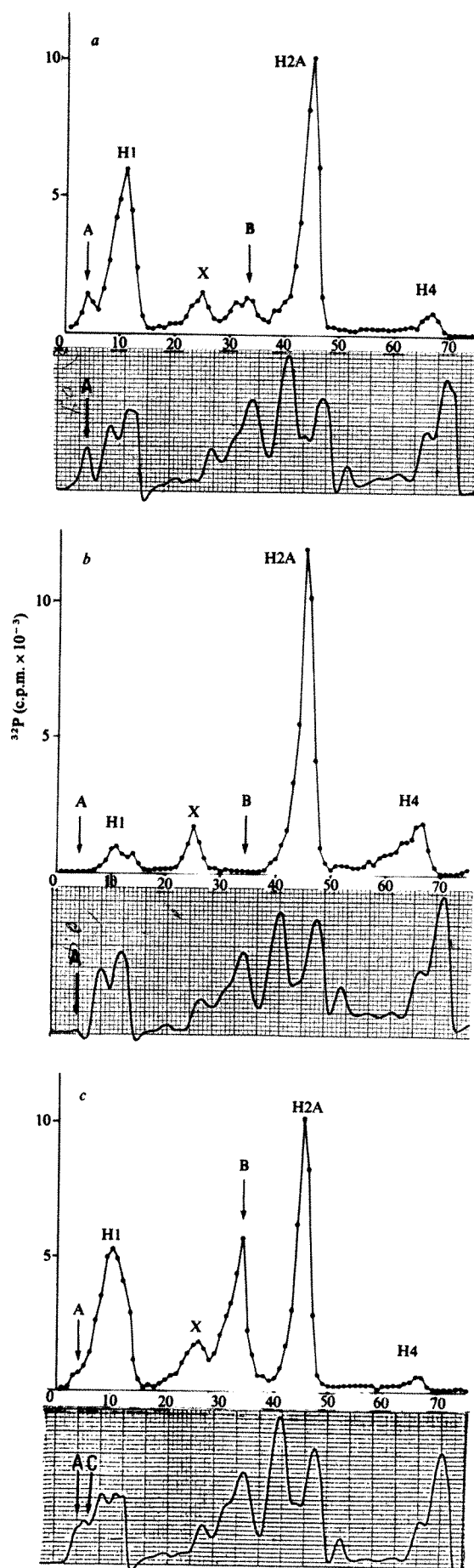


Fig. 3 The profile of phosphorylation of histones of ts85 cells at the permissive and non-permissive temperatures. The ts85 cells were synchronised at the G1/S boundary as described in Fig. 1 legend. When the cells were released from the blockade and entered the S phase, they were pulse labelled with ^{32}P -ortho-phosphate ($100\ \mu\text{Ci ml}^{-1}$, carrier free) for 2 h at 6 and 13.5 h after release from the blockade, corresponding to the G2 and M phases at 33 °C. The ts85 cells arrested at the G2 phase at 39 °C were labelled at 13.5 h. Histone fractions were extracted as follows. Cells frozen at -20 °C ($5\text{--}10 \times 10^6$ cells) were thawed and suspended in 1 ml of hypotonic buffer T (10 mM Tris-HCl, pH 7.8, 2 mM MgCl_2 , 0.5 mM EDTA, 2 mM 2-mercaptoethanol and 0.025% Triton X-100). After standing for 10 min at 4 °C, they were homogenised by 15 strokes with a Teflon pestle glass Potter Elvehjem homogeniser and centrifuged at 800g for 5 min. The precipitate was washed successively with buffer T, buffer A (50 mM Tris-HCl, pH 7.4, 0.14 M 2-mercaptoethanol and 50 mM sodium bisulphite) and buffer A containing 0.15 M NaCl, with stirring. Nuclei were pelleted at 12,000g for 10 min. Histones were then extracted by 0.4 N sulphuric acid for 1 h with stirring at 4 °C. Histones obtained were pelleted by 18% trichloroacetic acid and washed once with acetone-HCl (HCl 0.5% v/v) and twice with acetone. They were analysed by acid urea 15% polyacrylamide gel electrophoresis (0.9 N acetic acid-2 M urea, 25 cm gel) according to the method of Panyim and Chalkley²³. After electrophoresis for 30 h, the gels were stained with Coomassie brilliant blue R and were scanned with a densitometer. Gels were frozen at -80 °C and cut into 1.2-mm slices. Each slice was put into a vial, solubilised with Soluene 350 (Packard) and the radioactivity measured by a liquid scintillation spectrometer using toluene-based scintillation fluid. *a*, G2 phase at 33 °C; *b*, G2-arrested at 39 °C; *c*, M phase at 33 °C. A densitogram is shown under the profile of each phosphorylation pattern. X was the unknown protein which existed in wild-type cells and ts85 cells at both temperatures. The peaks which appeared in the densitogram, from left to right, correspond to H1, H3, H2B, H2A and H4 histone, respectively.

than at 33 °C. The highly phosphorylated form of the H1 histone which was detected in M phase cells (indicated by arrow C in Fig. 3c) did not exist in the G2-arrested cells at 39 °C. It has been reported that when the synthesis of histones is blocked by an inhibitor of DNA synthesis⁷ or X-ray irradiation²², the phosphorylation of all the histones is apparently reduced. In the ts85 cells, the amount of histones synthesised at 39 °C was 85% of that at 33 °C and the synthesis of H1 histone was not specifically inhibited at 39 °C. Furthermore, a band behind the H1 histone subfractions (as shown by arrow A in Fig. 3), was found at 33 °C but not at 39 °C. This protein is insoluble in 5% perchloric acid (PCA) and extracted by 0.2 M sulphuric acid. The role of this protein is unknown but it may be related to chromosome condensation. The specific decrease in the rate of H1 histone phosphorylation and the absence of the highly phosphorylated form of H1 histone at 39 °C did not result from cessation of the cell cycle at the G2 phase, because the rate of H1 histone phosphorylation remained constant throughout the S to M phases in both wild-type cells and grow-revertant ts85 cells. Other ts mutants (ts18 of FM3A cells, 11C3 of CHO cells) which were also arrested at the G2 phase at 39 °C had no defects in H1 histone phosphorylation (unpublished data).

Figure 3 also shows that H3 histone (indicated by arrow B) was not phosphorylated at 39 °C, whereas at 33 °C ts85 cells entered the mitotic phase and marked H3 histone phosphorylation occurred^{5,7,8}. This lack of H3 histone phosphorylation may be due to the cessation of the cell cycle, that is, ts85 cells may have been arrested before the onset of the mitotic phase.

The above data suggest that the phosphorylation of H1 histones is one of the factors involved in the initiation of chromosome condensation and the low level of H1 histone phosphorylation may induce incomplete condensation of the chromatin.

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The relationship between coding sequences and function in haemoglobin

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Gilbert has suggested that the presence of intervening sequences in DNA, called introns, can speed evolution by allowing novel proteins to be constructed from the pieces of existing ones¹. This hypothesis further suggests that the coding sequences, called exons, correspond to functional parts of the protein. The most striking example so far is the case of the immunoglobulin γ heavy chain, where the four polypeptide sequences corresponding to the four coding sequences form structurally and functionally distinct parts of the molecule^{1,2}. The relation between the three coding sequences of the β globin gene and structure or function is not as obvious, but the central coding sequence does code for the part of the globin chain which forms the haem crevice^{3,4}. To further test the idea that coding sequences correspond to functional units of proteins we consider the relationship between the coding sequences of α and β globin genes and the corresponding parts of the complete, tetrameric haemoglobin molecule.

The principal function of haemoglobin in vertebrates is to transport oxygen. Haemoglobin performs this function effectively by binding oxygen cooperatively, and with an overall affinity appropriate to physiological conditions. The affinity is regulated by protons and carbon dioxide (the Bohr effect), and by organic phosphates (2,3-diphosphoglycerate (DPG) in mammals). According to the simplest allosteric model for cooperative behaviour⁵, haemoglobin exists in two affinity states, corresponding to the quaternary structures of oxyhaemoglobin (R state) and deoxyhaemoglobin (T state). Binding within either the T or R state is noncooperative. Cooperativity is achieved by switching from the low affinity T state to the high affinity R state as successive molecules of oxygen bind. Regulation of oxygen affinity is achieved by the preferential binding of protons, carbon dioxide, and organic phosphates to the low affinity T state. The difference in tertiary conformation within

each quaternary structure produces a difference in affinity of the haems for oxygen. The model is somewhat oversimplified, but provides a good first order description of functional properties and a useful framework for correlating structure with function⁶⁻¹¹.

The large number of investigations on structure-function relationships in haemoglobin, which were pioneered by the work of Perutz and coworkers, make it possible to assign well defined functional roles to individual amino acid residues^{6,12,13}. From inspection of the three-dimensional structure of human haemoglobin, five functional categories are apparent^{6,11,14-16}.

(1) Residues in contact with the haem. These residues provide the hydrophobic environment necessary for the haem to be oxygenated by oxygen, rather than be oxidised. Haem contact residues also transmit structural changes, caused by oxygenation at the haem, to the $\alpha_1\beta_2$ subunit interface. (2) Residues in the $\alpha_1\beta_1$ contact. When haemoglobin switches quaternary structure, the $\alpha_1\beta_2$ intersubunit bonding is altered, but the same residues are involved in both quaternary structures. (3) Residues in the $\alpha_1\beta_1$ contact. This contact remains unaltered by the quaternary structure change. (4) Residues of the β chain that bind DPG. (5) Residues involved in the Bohr effect. As protons are not resolvable in the X-ray electron density map of haemoglobin, these residue assignments require detailed consideration of both the structure and solution chemistry^{6,11,17}.

Functional studies on haemoglobin variants provide support for the residue assignments. The functional residues, together with the known variants of human haemoglobin^{18,19} are listed in

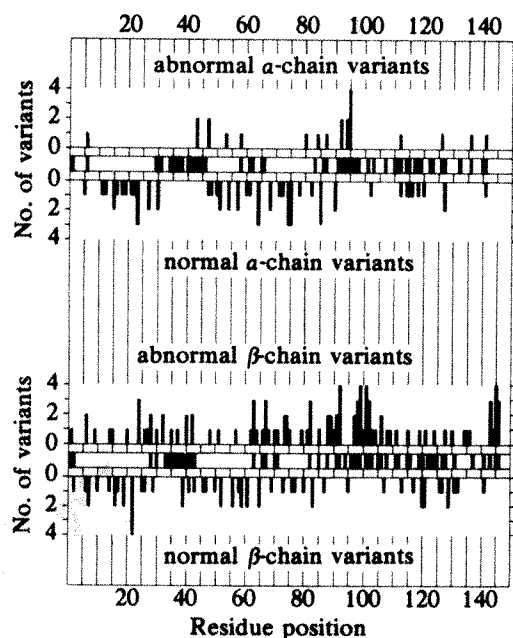


Fig. 1 Residues with well defined functional roles in human haemoglobin and α and β variants. For each chain, positions are marked for those residues that belong to one of the five categories discussed in the text. Haem contact, $\alpha_1\beta_2$ contact, and $\alpha_1\beta_1$ contact residues for deoxyhaemoglobin are taken from Fermi¹⁴. Haem contact, $\alpha_1\beta_2$ contact and $\alpha_1\beta_1$ contact residues for human carbomonoxyhaemoglobin, defined as a residue containing at least one atom within 4 Å of a haem atom or adjacent subunit atom, were calculated from the coordinates deposited by Joyce Baldwin with the Brookhaven Protein Data Bank. DPG binding site residues are taken from Arnott¹⁶; and Bohr effect residues are taken from Baldwin and Chothia¹¹ and Matthew *et al.*¹⁷. Carbon dioxide binds to α -amino groups of both α and β chains. Above the functional residues list are the number of variants at each residue position with known abnormal properties *in vitro*, and below the functional list are the number of variants at each residue position with no known abnormal properties *in vitro*. For the most part, the variants have not been studied in great detail. It is, therefore, likely that some variants listed as normal may exhibit functional impairment when examined further. The list of haemoglobin variants is published in the journal *Hemoglobin*^{18,19}.

Fig. 1. Among α chain variants with known abnormal properties *in vitro*, 77% (17 out of 22) contain substitutions at one of the functional residue positions, while 83% (48 out of 58) of the variants with no known abnormal properties *in vitro* contain substitutions at 'non-functional' residue positions. Among β chain variants the corresponding values are 63% (66 out of 104) and 83% (49 out of 59). If clinical findings are used as criteria, all four percentages are higher^{12,29}. These statistics support the relative significance of the residues in the five categories, but also suggest that functionally significant residues are missing from the list. For example, there are additional residues in the interior of the protein that maintain the stability of the three-dimensional structure, and additional residues that undergo conformational changes as part of the cooperative binding mechanism^{11,20}. Also, residues on the haemoglobin surface function to prevent aggregation of tetramers (as occurs in sickle cell disease) at the high concentrations found in the red cell²¹. Residue assignments for these functions cannot at present be made with confidence.

The residues associated with each of the five functions are listed in Fig. 2, which also shows their distribution in relation to the three coding sequences of the α and β globin genes. First, as pointed out by Blake³ and Gibert⁴, most of the residues (16 out of 20) in contact with the haem correspond to the central coding sequence of the β globin gene. In the α gene, 16 out of 20 of the haem contact residues are also contained in the central sequence. The distribution of intersubunit contacts among the three coding sequences shows a very strong correlation as well. In the case of the $\alpha_1\beta_2$ contact, the central sequence contains 12 out of the 15 residues in the β subunit that form intersubunit bonds, and 13 out of 18 residues in the α subunit. For the $\alpha_1\beta_1$ contact the third sequence contains 13 out of the 18 intersubunit bonding residues in the α subunit, and 14 out of 18 in the β subunit. The distribution of residues involved in binding the regulators—protons, carbon dioxide, and DPG—indicates no marked correlation, although the most important residues are contained in the first and third sequences.

The distribution of functional residues among the three coding sequences suggests that coding sequences do correspond to the major functions of haemoglobin. It is therefore interesting to speculate on the possible role that coding sequences played in the evolution of haemoglobin. One possible evolutionary pathway for mammalian haemoglobin is the following. The primitive haemoglobin is assumed to be a monomeric protein, capable of reversible oxygen binding, and coded for by a gene containing three separate coding sequences. Following gene duplication point mutations in the central coding sequence of the primitive α and β globin genes led to the development of the $\alpha_1\beta_2$ dimer that was capable of cooperative oxygen binding. (Note that lamprey haemoglobin, which has previously been considered to behave as a possible intermediate in the evolution of haemoglobin tetramers from monomers^{26,27}, is a monomer when oxygenated. It achieves cooperativity by aggregating in the deoxy state to form a homodimer, which is very probably of the $\alpha_1\beta_2$ type^{26,27}.) The maximum Hill n value for such a dimer was 2. An increase in cooperativity toward the current n value of 2.8 to 3.0 took place with the evolution of the $\alpha_1\beta_1$ contact to form a tetramer. The concentration of $\alpha_1\beta_1$ contact residues in the third sequence suggests that the third coding sequence in the α or β globin gene (or both) replaced its predecessor by recombination, thereby speeding the evolutionary process. The most recent events of evolution involved development of the Bohr effect and DPG binding residues, which are responsible for fine-tuning haemoglobin function. The almost complete absence of haem or intersubunit contacts in the first polypeptide sequence makes it conceivable that the corresponding coding sequence also replaced the preceding one in both α and β globin genes by recombination, thus more rapidly completing the evolution of the regulation of oxygen binding by protons, carbon dioxide, and DPG.

Considerations of haemoglobin evolution from amino acid sequence comparisons among species do not appear to argue

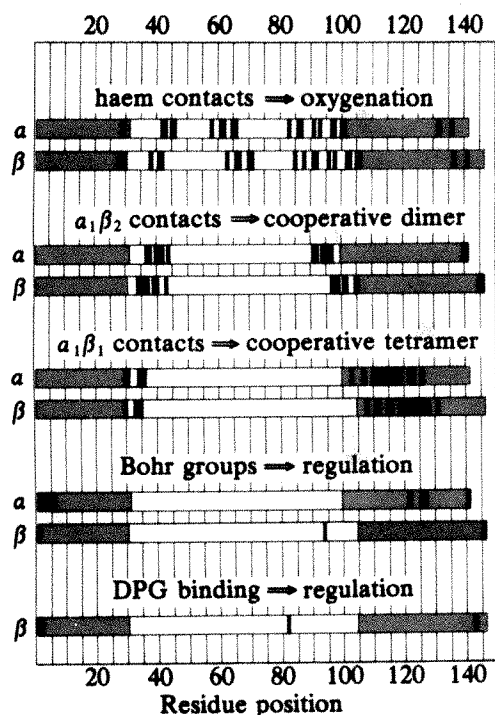


Fig. 2 Residues with well defined functional roles in human haemoglobin and the coding sequences of the mouse α and β globin genes. In the mouse α globin gene, the three coding sequences correspond to the amino acid residue positions 1–31, 32–99, and 100–141, (refs 22, 23) while in mouse β globin the sequences are 1–30, 31–104, and 105–146, which are structurally homologous to the α globin sequences^{24,25}.

strongly for or against this pathway in which the current first and third coding sequences arose from replacement of whole sequences in single steps. Goodman *et al.* have calculated that the number of nucleotide replacements per amino acid, from the point of gene duplication to the α and β chain amniote ancestors, was greater for the $\alpha_1\beta_2$ contact residues than for either the haem contact or $\alpha_1\beta_1$ contact residues¹⁵. In contrast, during the subsequent evolution to the current haemoglobins, the number of nucleotide replacements per position was greater for the $\alpha_1\beta_1$ contact residues than for either the haem or $\alpha_1\beta_2$ contact residues. If one takes the point of view that amino acid replacements signal the evolution of function at those positions, then these data support the idea that in the $\alpha_1\beta_2$ contacts evolved before the $\alpha_1\beta_1$ contacts.

The observation of a correlation between coding sequences and function presented here suggests that an investigation of the functional properties of the three polypeptide sequences of haemoglobin corresponding to the three coding sequences could provide new insight into gene-function relationships. Preparation of several of the sequences by digestion with trypsin is possible²⁸, since arginines are strategically located in both α and β chains, and in some haemoglobin variants^{18,19}.

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Isolation of chromosomal origins of replication in yeast

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Origins of replication have been identified in the DNA of viruses¹, mitochondria², bacterial plasmids³ and the bacterial chromosome⁴. However, origins of replication of eukaryote chromosomes have remained elusive because of the large size and sequence complexity of chromosomes and in particular for want of a suitable assay for their detection. Recent development of techniques for genetic transformation of yeast by autonomously replicating cytoplasmic plasmids^{5–8} now makes it possible to search for eukaryote origins in a manner analogous to that used for bacteria⁴. Here we describe the construction and properties of a plasmid which contains no effective eukaryote replication origin and whose efficiency of replication in yeast is greatly enhanced by insertion of certain fragments of yeast chromosomal DNA. We believe these to contain replication origins, since yeast transformants are shown to contain copies of the transforming plasmids.

The 13.7-kilobase recombinant plasmid pJDB248, consisting of the yeast 2 μ m plasmid, of pMB9 (a derivative of ColE1) and a 2.4 kilobase fragment of yeast DNA containing the *LEU2* gene, can be used to transform *Saccharomyces cerevisiae* M16, *leu2-3, his4-712^{FS}, ade2-1, lys2-1, SUF2*, to the *leu+* phenotype with a frequency of 10^4 – 10^5 transformants per μ g of DNA⁵. To obtain a plasmid whose presence in yeast can be detected, yet which lacks the origin of replication provided by the 2 μ m plasmid, a 1.5 kilobase *HaeII* fragment of pJDB248 containing the *LEU2* gene was recombined into the tetracycline resistance gene of the bacterial plasmid pBR325 (Fig. 1). The resultant 6.2-kilobase molecule, pDAM1, can transform a leucine-requiring strain of *Escherichia coli* (JA221) to *leu+* and to chloramphenicol resistance with equal efficiency.

However, the ability of pDAM1 to transform yeast (M16) to *leu+* is much impaired compared with pJDB248. pDAM1 gives rise to two clearly distinguishable types of yeast transformant (Table 1). One grows rapidly on leucine-free medium (1–10 transformants per μ g DNA) and probably results from recombination of pDAM1 or part of it with host chromosome¹⁰. The other is much more frequent (20,000 transformants per μ g DNA) but grows extremely slowly, the cells taking up to five days to form microcolonies visible to the naked eye. Cells from these colonies can be serially recombined on leucine-free medium, giving rise to further microcolonies, although the relative cloning efficiency is only 0.01–0.1% of that in the presence of leucine (Table 1). By contrast, pJDB248 transformants have a relative cloning efficiency of 78%. Furthermore, these pDAM1 transformants produce no visible turbidity after inoculation of a 2 ml leucine-free liquid culture even after a week. These data are taken to indicate that pDAM1 can survive and express the

LEU2 gene in yeast, but that the rate of growth of these transformants is limited by the very low copy number of the plasmid, perhaps no more than one molecule per 1,000–10,000 cells, resulting from the failure of pDAM1 to replicate.

It is possible to insert fragments of yeast DNA into pDAM1 and screen for those which give rise to yeast transformants which grow rapidly in the absence of leucine. Yeast whole cell DNA was digested with *Pst*I, ligated with the *Pst*I site within the ampicillin gene of pDAM1, and the total mixture cloned in *E. coli*. DNA preparations were made from 107 separate bacterial colonies and each was tested for its ability to transform yeast to *leu*⁺. Of such preparations 99 gave rise to microcolony transformants, indistinguishable in growth properties from those of pDAM1 itself, whereas 8 (pDAMY1 to 8) gave rise to colonies all of which grew rapidly, similarly to pJDB248 transformants (Table 1). Analysis by restriction digestion of these 8 DNA preparations showed two—pDAMY5 and pDAMY6—to contain the full length yeast 2 μ m plasmid which is endogenous to the strain (M16) from which the yeast DNA was prepared, and has one *Pst*I restriction site. The remaining 6 have *Pst*I inserts into pDAM1 ranging in size from 0.7 to 6.3 kilobases which are presumed to derive from the yeast chromosomal DNA (Table 1).

To determine whether copies of these plasmids are present in yeast transformants, lysates of single yeast colonies were prepared and the DNA analysed by agarose gel electrophoresis. The DNA was transferred to nitrocellulose filters and hybridised to ³²P-labelled pBR325 (Fig. 2). Tracks 1 and 2 of the autoradiograph contain DNA from the untransformed host strain (M16) and a low frequency transformant of pDAM1. Neither of these shows hybridisation to pBR325 detectable in this experiment. A high frequency transformant of pDAM1 was not tested as these colonies do not grow adequately on leucine-free media. However, transformants of pDAMY1, pDAMY2 and pJDB248 (tracks 3–8) have bands, hybridising to pBR325, with electrophoretic mobilities corresponding to the supercoiled (rapidly migrating) and open-circular (slower migrating) forms of the transforming plasmids. The presence of plasmids hybridising to pBR325 has been found in transformants of each of pDAMY1–8 (data not shown).

Why does insertion of certain fragments of yeast DNA into pDAM1 greatly increase the ability of transformants of this

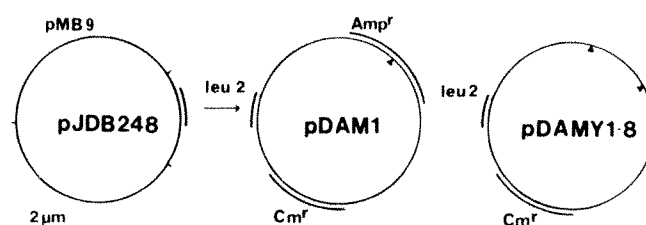


Fig. 1 Illustration of the relationship between the plasmids pJDB248, pDAM1 and pDAMY1–8. pJDB248 consists of pMB9, the yeast 2 μ m plasmid and a 2.4-kilobase insert complementing the yeast *leu2* mutation. pDAM1 consists of pBR325 with a 1.5-kilobase insert containing the *LEU2* gene. This plasmid also carries chloramphenicol resistance (*Cm*^r) and ampicillin resistance (*Amp*^r). pDAMY1–8 are derived from pDAM1 by insertion of *Pst*I fragments of yeast DNA into the *Pst*I site (Δ) of the ampicillin gene of pDAM1. To construct pDAM1, pBR325 purified by the cleared lysate procedure⁹ and caesium chloride centrifugation was partially digested with the restriction endonuclease *Hae*II and ligated with a partial digest of pJDB248. The ligated DNA was used to transform *E. coli* JA221 *recA1*, *leuB6*, *trp* Δ *ES*, *hsdR*[–] *hsdM*⁺ *lacY* C600, to *leu*⁺ using minimal selective plates. pDAM1 was one such transformant, consisting of a 1.5-kilobase *Hae*II fragment containing the *LEU2* gene inserted into the tetracycline resistance gene of pBR325. Approximately 0.75 kilobases of this gene are absent. Yeast DNA was prepared from the strain M16 by lysis of protoplasts⁵, phenol extraction and ethanol precipitation. The DNA was digested with *Pst*I and ligated with a *Pst*I digest of pDAM1. This DNA was used to transform JA221 to chloramphenicol resistance and ampicillin sensitive colonies containing inserts of yeast DNA were subsequently selected.

plasmid to grow in the absence of leucine? It is most unlikely that this phenomenon is related to the efficiency of expression of the *LEU2* gene. This gene derived from yeast and carried in pDAM1 is expressed adequately in *E. coli*. Equally, it is unlikely that the result is a consequence of the ability of certain fragments of yeast DNA to allow vastly more efficient integration of pDAM1 into the yeast chromosome. Such integration may occur but it does not readily account for the presence of free plasmid copies in transformants. We believe that the poor growth of pDAM1 transformants is due to a very low copy

Table 1 Properties of pDAM1, its derivatives, and the yeast transformants which they produce

Plasmid	Size (kilobases)	Transformation frequency colonies per μ g DNA	Relative cloning efficiency <i>leu</i> [–] / <i>leu</i> ⁺ (%)
pDAM1	6.2 A	1–10	98
	B	20,000	0.01–0.1
pDAMY1	10.5	10,000	32
pDAMY2	11.2	10,000	50
pDAMY3	12.5	10,000	25
pDAMY4	9.1	10,000	65
pDAMY5	12.0	10,000	12
pDAMY6	12.0	10,000	8
pDAMY7	12.4	10,000	9
pDAMY8	6.9	10,000	2

The plasmids pDAMY1 to 8 are derived from pDAM1 as described in the legend to Fig. 1. Their size is given in kilobases and the approximate frequencies with which they transform the yeast strain M16 to *leu*⁺ are also given. The methods for yeast transformation was as previously described⁵. The relative cloning efficiency of yeast transformants was obtained as follows. Transformant colonies were spread on leucine-free minimal plates and allowed to grow overnight at 29 °C. Approximately 1,000 cells were suspended in 0.5 ml H₂O, aliquots of 100 μ l were spread onto leucine-containing and leucine-free minimal plates. Colonies were counted after 2 days except that pDAM1 microcolonies required 5 days to grow. The relative cloning efficiency is the ratio of the number of colonies on *leu*[–] plates to that on *leu*⁺ plates expressed as a percentage.

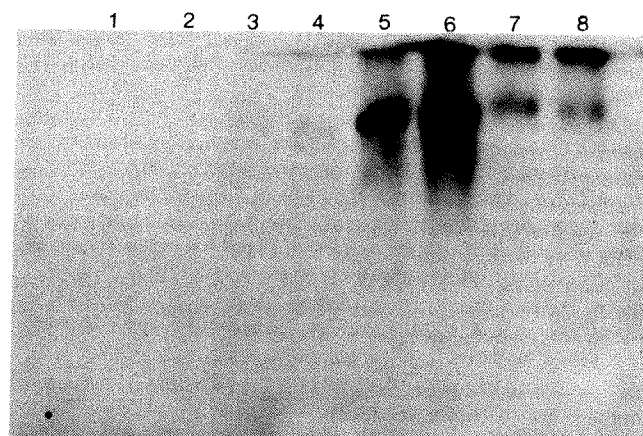


Fig. 2 Autoradiograph of ³²P-labelled pBR325 hybridised to undigested DNA of yeast transformants. Track 1: untransformed recipient strain M16. Track 2: rapidly growing transformant of pDAM1. Tracks 3,4: transformants of pDAMY1. Tracks 5,6: transformants of pDAMY2. Tracks 7,8: transformants of pJDB248. DNA was prepared from transformant colonies⁵, which had been grown on leucine-free media, and electrophoresed through 0.8% Agarose at 3 V cm^{–1} for 15 h. DNA was transferred to a nitrocellulose filter¹⁰ and hybridised with 4 \times 10⁶ d.p.m. ³²P-labelled pBR325 prepared by nick translation. The filter was exposed to Fuji Rx film with a Kodak intensifying screen at –60 °C for 4 days.

number of the molecule resulting from a lack of a eukaryotic origin of replication. The little replication that certainly does occur may be due to utilisation of the bacterial origin contained in pBR235. Those derivatives of pDAM1 which allow rapid growth of transformants are thought to be present in a higher copy number than pDAM1 because they contain origins of replication. This view allows an estimation of the frequency of origins in the yeast chromosome. Of 105 *Pst*I fragments of yeast DNA tested (excluding those two shown to be the 2 μ m plasmid), 6 contain origins. As the average size of the *Pst*I inserts in our clones is 2.3 kilobases there is, on average, one origin in every 40.2 kilobases of chromosome. This compares with the figures of 68 and 90 kilobases obtained respectively by electron

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microscopy and fibre autoradiography of replicating yeast DNA^{12,13}. It remains to be established whether the apparent origins of replication detected in our system are indeed those utilised during normal chromosomal DNA replication.

Our work allows further isolation and subsequent sequence analysis of any desired number of yeast chromosomal replication origins. It is hoped that information about the nature of replication origins will aid studies of the biochemical mechanism of initiation of eukaryote DNA synthesis.

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Is 3'-nucleotide rigid?

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Conformational analysis of nucleic acids and polynucleotides is far more complex than that of proteins and polypeptides, due to five single bond rotations in addition to the sugar puckerings in the monomer. Sundaralingam¹ proposed the concept of the 'rigid' nucleotides from analysis of crystal structure data, with the flexibility allowed only about the phosphodiester bonds. However, the crystal structure of deoxyguanosine-5'-phosphate^{2,3} indicates at *gt* conformation about the C-4'-C-5' bond against *gg* in a conformationally rigid nucleotide¹. Jack *et al.*⁴ considered the flexibility of nucleotides in tRNA about the C-4'-C-5' bond, thereby introducing the concept of 'non-rigid' ribonucleotides. Conformational flexibility of the furanose ring in DNA and RNA and their energetics using classical and quantum chemical methods have been reported⁵⁻⁸. We have examined the flexibility of 3'-nucleotides. α , the most important of the conformational parameters defining the 3'-end of a nucleotide unit⁹, has a value in the range 195°-270° in all the 3'-nucleotides, dinucleoside monophosphates and higher oligomers which have been surveyed. A survey of the proposed structures of polyribonucleotides^{10,11} also shows the values of α to be greater than 200°. However, the structures proposed for B-DNA by Arnott and Hukins^{12,13} and D-DNA by Arnott *et al.*¹⁴ have values of α of 155° and 141° respectively, much lower than the lowest observed value. The structure for B-DNA has two strong, short contacts (C-2' ... OP-1 = 2.64 Å and HC-2' ... OP-1 = 1.79 Å) which lead to an energetically unfavourable conformation. Hence, it is of interest to investigate whether, by allowing flexibility to the sugar moiety in the nucleotide unit, it is possible to make the structure energetically favourable. Here, conformational energy calculations were carried out to determine the range of α which would give rise to energetically favoured conformations with different sugar puckerings. Our analysis has shown that the theoretically obtained range is nearly the same as the preferred range in crystals, indicating the flexibility of the 3'-nucleotides.

Conformational energy calculations were carried out using classical potential functions¹⁵ for the sugar-phosphate fragment (Fig. 1a) of the nucleotide unit for both ribose and deoxyribose sugars. The sugar geometries were chosen from the minimum energy regions of the pseudo-rotation space^{7,8}. For each of the

values of Φ (defined in Fig. 1b legend), energy was minimised for each value α over a few geometries. Φ was varied from 0° onwards at intervals of 18° , and α from 120° to 300° at intervals of 10° and 1° (in the minimum energy region). Outside the specified range for α , for all geometries considered, strong short contacts were observed between the atoms in the phosphate group and the groups of atoms attached at the C-5' and C-2' atoms. The torsion angles β and ϵ were fixed respectively at their average values of 290° and 50° (ref. 16). The other two pendant oxygens were fixed tetrahedrally. Energies were also obtained for *gt* and *tg* conformations about the C-4'–C-5' bond. The general features of the Φ – α plot do not change for small changes from the average values of β and ϵ . However, for the *tg* conformation, it was observed that the C-3' *endo* region does not have energetically favourable conformations for both deoxyribose- and ribose-phosphate fragments. Details of this will be published elsewhere.

Figure 2 shows the energy contours at an interval of 1 kcal mol⁻¹ from the minimum in the Φ - α space for the ribose-phosphate fragment. Observed values for α in many crystal structures are also plotted. The plot shows the global minimum

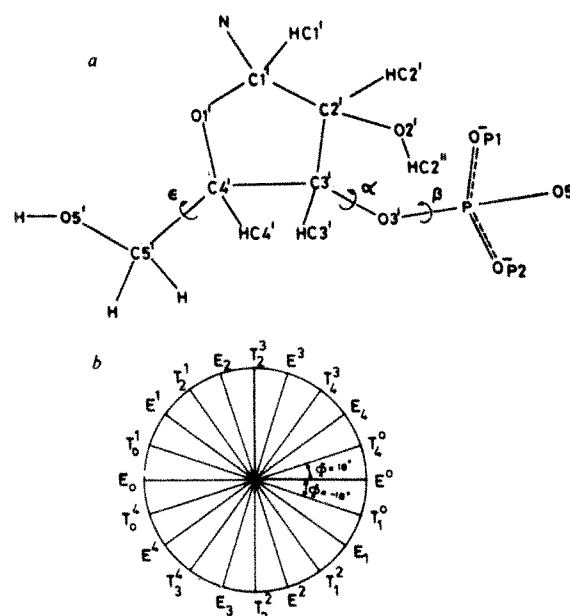


Fig. 1 *a*, The ribose-phosphate fragment of a ribonucleotide. *b*, Definition of the pseudo-rotation phase parameter in the pseudo-rotation space.

at $\alpha = 280^\circ$ in the C-2' *endo* region. The minimum has been indicated by a vertical line, as, along this line energy does not vary significantly. The energy varies differently in two directions about the minimum. On the left ($\alpha < 280^\circ$), the energy rises very gradually as α decreases. On the right ($\alpha > 280^\circ$), as α increases, the energy increases very rapidly due to strong unfavourable non-bonded interactions between atoms in the phosphate group and hydroxyl group and hydrogen atoms at C-5'. Apart from the global minimum at $\alpha = 280^\circ$ in the C-2' *endo* region, the plot also shows two local minima in the C-3' *endo* region at $\alpha \approx 200^\circ$ and $\alpha \approx 270^\circ$. Note also that transitions are possible from the C-3' *endo* to C-2' *endo* regions via the O-1' *exo* region at high values of α by crossing an energy barrier of 1 kcal mol⁻¹.

Figure 3 shows a similar plot for the deoxyribose-phosphate fragment. As in ribose, there is a global minimum in the C-2' *endo* region at $\alpha = 280^\circ$ (indicated by a vertical line for reasons stated above). Also as in ribose and for reasons already stated, this plot indicates a rapid rise of energy as α is increased to the right of the global minimum. To the left ($\alpha < 280^\circ$) there is a gradual rise in the energy. However, unlike in ribose, there is another local minimum around $\alpha \approx 190^\circ$ in the C-2' *endo* region in addition to two local minima in the C-3' *endo* region (at $\alpha \approx 180^\circ$ and $\approx 270^\circ$). The plot also shows that conformational transitions between the C-3' *endo* and C-2' *endo* regions are possible via both O-1' *exo* and O-1' *endo* regions. At low values of α ($\approx 200^\circ$) the barrier to transition via either O-1' *exo* or O-1' *endo* is 2 kcal mol⁻¹. At high values of α ($\approx 270^\circ$) the barrier via O-1' *exo* is 1 kcal mol⁻¹ and is very high via O-1' *endo*.

A comparison of the Φ - α plots for ribose- and deoxyribose-phosphate fragments shows a larger conformational flexibility in the case of the latter. At low α values ($\approx 180^\circ$), C-2' *endo* region is prohibited for ribose because of strong short contacts between the bulky OH group at C-2' of the sugar and the atoms in the phosphate group. These are relieved to some extent in deoxyribose, where only hydrogen is attached at C-2'. This explains the occurrence of a local minimum at $\alpha \approx 190^\circ$ in the C-2' *endo* region. However, for both the sugars, we noted that in both the C-3' *endo* and C-3' *exo* regions, very low values of α , such as 140° - 150° , are prohibited. Energy contours as high as 4-5 kcal mol⁻¹ pass through the regions at such low α values. In the C-3' *endo* region the local minimum at $\alpha \approx 200^\circ$ in the case of ribose shifts by about 20° on the lower side in the case of deoxyribose; this is due to the presence of hydrogen at C-2' instead of a bulky hydroxyl group. The comparison also reveals that transitions between C-3' *endo* and C-2' *endo* regions are very restricted in ribose, but are relatively easy in deoxyribose. Whereas in ribose only high α transitions are possible via the O-1' *exo* region, both low and high transitions via the O-1' *exo* and O-1' *endo* regions are possible for deoxyribose.

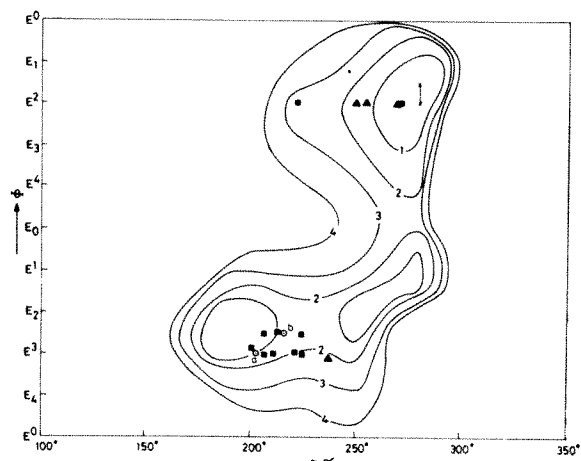


Fig. 2 Φ - α plot for the ribose-phosphate fragment. Energy contours of 1.5 kcal mol⁻¹ in the C-3' *endo* region are shown. \blacktriangle Denotes 3'-nucleotides, \blacksquare denotes dinucleoside monophosphates and higher oligomers, \odot denotes proposed structures of polynucleotides. \odot^b , RNA-11 (ref. 11); \odot^a , A-RNA (ref. 10).

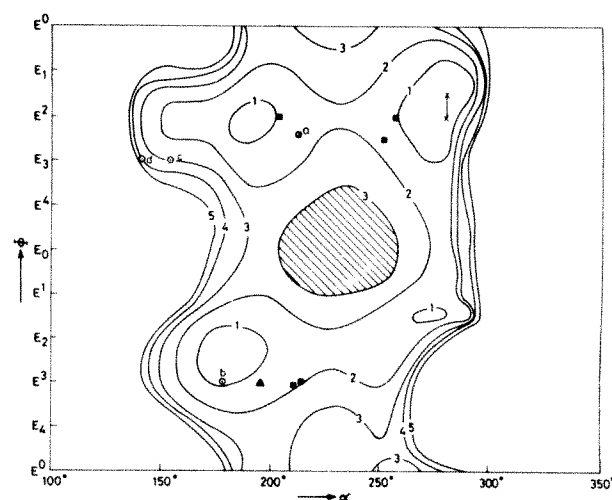


Fig. 3 Φ - α plot for the deoxyribose-phosphate fragment. \blacktriangle Denotes 3'-nucleotides, \blacksquare denotes dinucleoside monophosphates and higher oligomers, \odot denotes proposed structures for polynucleotides. \odot^a , C-DNA¹⁸; \odot^b , A-DNA¹⁷; \odot^c , B-DNA^{12,13}; \odot^d , D-DNA¹⁴.

It is found that for compounds containing ribose sugars, values of α mostly range between 200° and 240° in the C-3' *endo* region and between 220° and 270° in the C-2' *endo* region. Two of the proposed structures for polynucleotides (A-RNA and RNA-11)^{10,11} also lie in the C-3' *endo* region. In the case of deoxyribose sugar compounds, the observed points are spread over a wider range of α than in ribose in both C-3' *endo* and C-2' *endo* regions. It is found from the plots that energetically favourable conformations are possible with $\alpha > 180^\circ$, and that for structures with $\alpha < 150^\circ$ unfavourable conformations result. The refined model of B-DNA¹³ ($\alpha = 155^\circ$) lies on the 3 kcal mol⁻¹ contour in the C-3' *exo* region of the Φ - α plot for the deoxyribose-phosphate fragment. However, β and ϵ in the refined structure of B-DNA are different from those used in the present calculations. Conformational energy calculations carried out using the conformational parameters of the refined model reveal that the model is 4 kcal mol⁻¹ above the minimum and thus energetically unfavourable. Further, V.S. has pointed out that the structure is 2 kcal mol⁻¹ above the minimum in the Φ - χ plot⁷, which emphasised the influence of the sugar geometry on the value of χ , the glycosidic torsion angle. Considering the nucleotide unit as a whole, the refined model of B-DNA¹³ is more than 5 kcal mol⁻¹ above the minimum despite allowance for the flexibility of the sugar unit.

We have used these results to produce a refined model for B-DNA which differs from that of Arnott and Hukins¹³. We have refined a right-handed double-helical model which is in good agreement with the X-ray diffraction data and has an energetically favourable conformation. We also find that the left-handed double-helical model for B-DNA is energetically favourable and is in good agreement with the X-ray diffraction data. The details of the two structures are published elsewhere.

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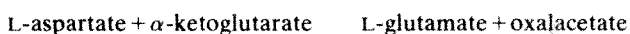
Electron density map of chicken heart cytosol aspartate transaminase at 3.5 Å resolution

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Aspartate transaminase (EC 2.6.1.1., Asp-transaminase) has been studied extensively, and much is now known about its physico-chemical, catalytic and other properties¹. X-ray studies that can provide a structural foundation for the events that occur during the transamination reaction are under way on three species of Asp-transaminase: the cytosolic enzyme from pig² and chicken³ hearts, and the mitochondrial chicken heart enzyme⁴. We describe here the interpretation of an electron density map of Asp-transaminase from chicken heart cytosol at 3.5 Å.

Aspartate transaminase catalyses the reaction



The enzyme is dimeric (molecular weight 93,000), consisting of two chemically identical subunits. Crystals were grown in the presence of α -methyl aspartate (α -Me-Asp) as previously described³. Their space group is $P2_12_12_1$, with cell dimensions $a = 62.7$ Å, $b = 118.1$ Å and $c = 124.5$ Å, and they contain one dimer in the asymmetric unit⁵. The 3.5 Å resolution map was calculated using phases obtained from three isomorphous derivatives, improved by making use of the non-crystallographic symmetry relating the two subunits in the molecule⁶. Other states of the enzyme were obtained by soaking experiments, and examined at lower resolution using difference maps.

Tracing the polypeptide chain was facilitated by the extensive secondary structure present in each subunit. Nine α -helices were located, including one 48 Å long, and a number of β -strands, five of which form a parallel β -sheet in the core of the subunit. Figure 1 shows part of the electron density map including the long helix and part of core β -sheet. It was possible to interpret the electron density map in terms of a unique chain tracing, a schematic drawing of which is shown in Fig. 2. According to the current classification of globular proteins⁷, Asp-transaminase is an α/β protein. Figure 2 shows that the subunit is composed of three parts of about equal size, defined by the preferential directions of the α -helices and β -strands. The three directions are almost mutually perpendicular. Of these three parts, only one, shown in the lower centre of Fig. 2, can be considered as a separate domain, the others are combinations of fragments from the N and C termini. The separate domain shows a close resemblance to the well-known 'nucleotide binding domain', first discovered in the NAD-dependent dehydrogenases^{8–11}, and then found in a number of other enzymes including phosphoglycerate kinase¹², phosphoglycerate mutase¹³, rhodanese¹⁴ and glycogen phosphorylase^{15,16}. In Asp-transaminase this domain is composed of five parallel β -strands alternating with five antiparallel helices. Figure 3 shows a drawing of this domain in comparison with the NAD-binding domain of lactate dehydrogenase^{17,18}. Interestingly, both pyridoxal phosphate-containing enzymes studied by X-ray analysis (Asp-transaminase and glycogen phosphorylase) have such a domain in their structure; other pyridoxal enzymes may also contain domains of this kind.

The enzyme's active site was first located from a difference map of the apoenzyme at 5 Å resolution³. At 3.5 Å resolution it can be concluded that the negative density seen in the difference map between the native and apoenzyme corresponds only to the pyridine moiety of the coenzyme; the phosphate moiety is probably replaced by a dianion from the solvent in the apoenzyme crystals. The phosphate group of the coenzyme has been recognised in the 3.5 Å native map as the highest density peak located close to the previously identified pyridine ring. The coenzyme is located at the carboxy end of the β -sheet of the nucleotide-binding unit, with its phosphate group at the N terminus of the first upper helix as shown in Fig. 2, suggesting an interaction of the charged phosphate with the helix dipole¹⁴. The coenzyme is located about 7 Å from the subunit interface, represented by the upper face of the drawing in Fig. 2. The substrate binding region, deduced from a series of difference maps (see below) is placed between the coenzyme and chain segments from the N and C termini (see Fig. 2). Overall the active site is located at the interface of the three parts of the subunit noted above.

Spectroscopic studies on crystals of pig cytosolic enzyme¹⁹ suggested that the complex with α -Me-Asp is a mixture of two spectroscopically discernable forms, probably representing differences in the coenzyme orientation. However, in the X-ray studies the electron density for α -Me-Asp can be accounted for by a single conformation and a fixed geometry of the substrate analogue in both forms can be assumed. The density of the pyridine ring is slightly less pronounced but the span of possible orientations is only 15–20°. We have shown previously³ that these crystals of Asp-transaminase are catalytically competent, allowing us to examine different states of the enzyme. Previous studies³ have shown that crystals washed free of α -Me-Asp, and colourless crystals of the pyridoxamine form of the enzyme gave 5 Å resolution difference maps containing similar features interpreted as conformational changes in the enzyme. Now it can be seen that these changes are located in a wide region including the substrate-binding area and the C-terminal helix. It seemed possible that the conformational pattern in this area is determined by the presence of substrate carboxyl groups. To check this the complexes with erythro- β -hydroxyaspartate, maleate and succinate have been studied.

Crystals were soaked in a stabilising solution³ (without α -Me-Asp) with the addition of one of the three mentioned

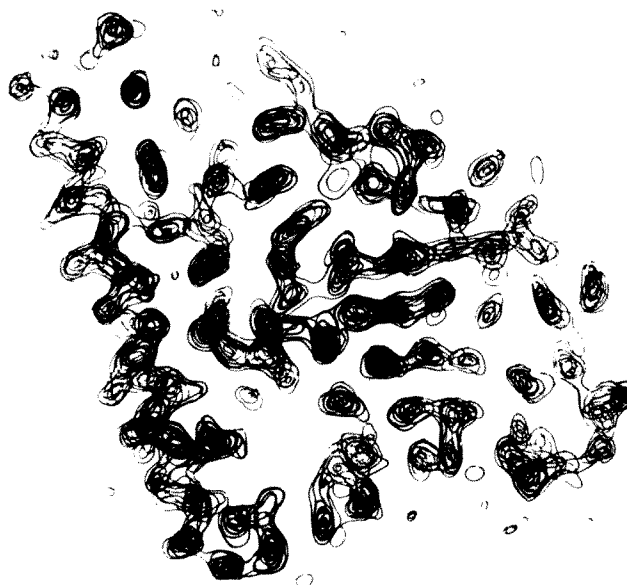


Fig. 1 Superposition of the eight sections of the electron density map of Asp-transaminase from chicken heart cytosol (at 3.5 Å resolution). The sections are perpendicular to the z axis, with the distance of 0.8 Å between neighbouring sections. A big helix 48 Å long is seen at left side. The horizontal β -strands of a parallel β -sheet in the centre are a part of the 'nucleotide-binding domain'.

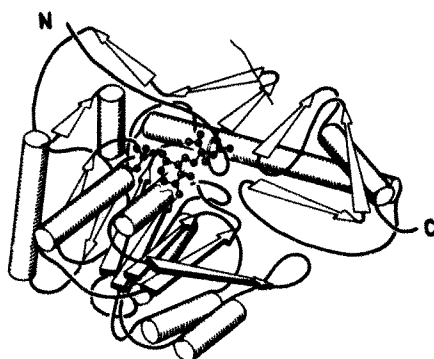


Fig. 2 Schematic drawing of the model of Asp-transaminase subunit built from secondary structure elements. α -Helices are shown as cylinders and β -strands as arrows, those belonging to the 'nucleotide-binding domain' shown as space-filled arrows. The coenzyme is shown covalently linked to α -Me-Asp.

compounds, at 50 mM concentration. Crystals soaked with erythro- β -hydroxyaspartate acquired a gingerish colour after 1 hour, which could be maintained for several months. The X-ray experiments were performed on crystals soaked for 2 h. Crystals soaked with maleate or succinate became a distinctly brighter yellow than the original α -Me-Asp complex. X-ray experiments were done after 1–2 days of soaking. At 5 Å resolution, the difference maps of all three complexes contained some features in the substrate-binding area but no conformational changes. A similar X-ray experiment on the abortive complex of the pyridoxal form of the enzyme with 2-oxoglutarate gave a difference map showing conformational changes localised in the same region as the substrate-free form of the holoenzyme³, but of opposite sign.

It is of interest that the conformations of the apoenzyme and the α -Me-Asp complex are similar. A number of prominent positive peaks observed near more extended features interpreted as conformational changes, suggest that some of the conformational changes can be ascribed to the interaction of ions. After the removal of α -Me-Asp one such positive peak persists near a position previously occupied by the distal carboxyl. Another is seen in the vicinity of the C-terminal helix. It can be supposed that an anion replacing the substrate carboxyls is a cause of the conformational changes in the substrate-binding area. Also, in relation to the apoenzyme, the absence of negative density for the removed phosphate of the pyridoxal phosphate or substrate analogue may be explained by similar anion replacements. It is possible that the additional space created by removal of the coenzyme allows the anion to bind without causing conformational distortions to the adjacent protein framework.

In the study of Asp-transaminase much attention has been paid to the so-called syncatalytic phenomena observed after the addition of pairs of substrates (such as the L-glutamate/2-

oxoglutarate pair)^{20–24}. These effects have been treated in terms of conformational changes concomitant with the formation of covalent intermediates in the course of the catalytic process. It is tempting to assume that the conformational changes discussed here are a part of the same picture. However some remarks seem to be relevant: (1) The conformational changes observed by us are concomitant not with catalysis but with binding of substrates or inhibitors. (2) The conformational changes that accompany the transition between substrate-binding, and substrate-free forms are different in two subunits, this asymmetry may be explained by restrictions imposed by crystal packing. (3) The possibility that the observed changes are a property only of the chicken heart cytosol enzyme cannot be excluded. (4) In addition to the gross conformational changes observed at 5 Å resolution, more subtle changes are probably also present which may be of the most important functional meaning. It may be predicted that comparative X-ray studies of different states of Asp-transaminase at higher resolution should soon clarify many of the problems discussed here.

We thank Yu. V. Nekrasov, A. A. Vagin and V. M. Kochkina for valuable assistance, and L. N. Johnson for the picture of 'nucleotide-binding domain' in phosphorylase *b*. We also thank Professor A. E. Braunstein for valuable advice and discussions. *Note added in proof:* Recently similar three-dimensional structure and conformational changes have been described for chicken mitochondrial Asp-transaminase²⁵.

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Corrigendum

In the News and Views item 'Structure and force generation in muscle' by J. M. Squire, *Nature* **281**, 99, lines 14 and 15 in the right-hand column should read '... of 20% of the actin monomers in the thin filaments or to 40% labelling...' (The values 20 and 40% were transposed in the original.)

Erratum

In the review article 'Nuclear weapons and power-reactor plutonium', by A. B. Lovins, *Nature* **283**, 817–822, p. 818 paragraph 1 line 4, should begin '(GWt-d)', paragraph 2 line 6 should read '... 27–33 GWt-d/T...'. A line was omitted from the paragraph on p. 822 headed 'Conclusions. Lines 10–17 should read: "and "not a valid concept"³¹. (Dilution with UO₂ or other materials requiring chemical or physical⁵⁶ separation is a valid concept—it means more material must be diverted and processed^{19,25,34}—but does not solve the problem.) Taking all effects on weapons physics into account, a high^{238,240,242}Pu content may reduce expected yield to a level that could devastate only a modest portion of a city rather than all of it, and may make that yield much less predictable, if the bomb is crudely made. But...' In refs 27 and 55 the title should read 'Nuclear Policies: Fuel Without the Bomb'.

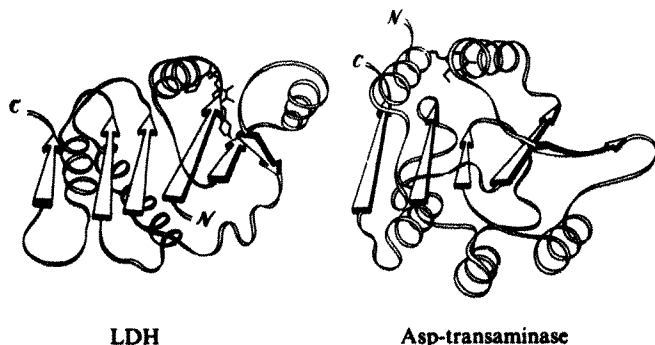


Fig. 3 Comparison of two domains present in the three-dimensional structure of lactate dehydrogenase and Asp-transaminase.

MATTERS ARISING

Information transmission in remote viewing experiments

IN a recent letter to *Nature*¹ Marks and Kammann offer criticism of the SRI experiments in 'remote viewing', the ability of certain individuals to access and describe, by means of mental processes, information blocked from ordinary perception by distance or shielding^{2,4}. They hypothesise that the apparent success in these experiments may simply be an artefact of statements in the subjects' transcripts which provide extraneous cues useful to judges attempting to blind match transcripts to target sites. They then argue that examples from the transcripts of the first published experiment—a nine-trial series with subject Price²—support their hypothesis. We present here experimental evidence that demonstrates that this conjecture is false.

On reading Marks and Kammann, one of us (C.T.T.), who had no connection with the original Price series, offered to independently reanalyse that series to test the validity of the Marks–Kammann hypothesis. He edited the transcripts carefully, removing all phrases suggested as potential cues by Marks and Kammann, and removing any additional phrases for which even the most remote *post hoc* cue argument could be made. The series was then rejudged by a new independent qualified judge (having previously shown competence in blind matching and verbal content analysis of similar materials) who was unfamiliar with the Price series. The materials turned over to the judge consisted of the newly edited transcripts presented in random order, and the list of target sites, also in random order (different from both the transcript random order and from the order of original target usage). The judge was instructed to provide, on a blind basis, a set of target/transcript correspondence ratings, which required that she visit each target site and rate transcripts to targets on a scale of 0–100 for all possible combinations, generating a 9×9 matrix. These data also yield the conventional overall measure of target/transcript correlations by indicating each first place match from the set of nine for each target.

The result of the blind matching of transcripts to target sites in the rejudging was that seven of the nine were again correctly matched. The appropriate statistic for this overall matching result is derived assuming non-independent assignment of transcripts to target sites (as in guessing the order of a random sequence of the digits one through nine, each used once)⁵; the result (seven out of nine correctly matched) is significant at

$P < 10^{-4}$. Furthermore, the more detailed target/transcript rating matrix was analysed by an exact factorial method⁶; the result obtained by this analysis was significant at $P = 2.2 \times 10^{-5}$. The greater significance obtained by the latter, more sensitive measure of target/transcript correlations reflected the following important fact: the judge found that, with the exception of two transcripts that did not seem to correspond to any site, the remaining seven transcripts each showed high correlation to one (correct) site and low correlation to the others. This is in direct contradiction to the implication of Marks and Kammann that little target/transcript correlation existed, and that matching is possible only on the basis of artefactual cues.

Therefore, on the basis of an independently conducted empirical test, we reject as invalid the Marks–Kammann conjecture that success in the first-published study on remote viewing is to be attributed to cueing artefacts rather than to transcript/target correlations. It is also important to note that the Marks–Kammann critique did not address the quality of the remote-viewing descriptions in the transcripts *per se*, but was instead limited to criticism of a particular judging procedure used to evaluate those descriptions. With regard to the descriptions themselves, we note that in the nine-transcript series in question, when the target was a boat marina the subject gave a consistent narrative that began with "What I'm looking at is a little boat jetty or boat dock along the bay. It is in a direction about like that (pointing) from here. Yeah, I see the little boats, some motor launch (sic), some little sailing ships. . . ." For a landmark Hoover Tower site, the subject summarised his impressions as "The area—I have a place—seems like it would be Hoover Tower". For a recreational swimming pool site with a $75 \text{ ft} \times 100 \text{ ft}$ rectangular pool and a 110 ft diameter circular pool, the subject made a drawing of the target area as centred about two pools of water, which he dimensioned as a $60 \text{ ft} \times 89 \text{ ft}$ rectangular pool and a 120 ft diameter circular pool; and so forth. Furthermore, as pointed out above, blind content analysis of the transcripts, which provides a sensitive measure of the degree of target/transcript correspondences, confirms objectively the subjective impression of above-chance correspondence that one infers from examples such as the above. With data of this quality we would argue that it is not surprising that empirical test failed to confirm the cueing-artefact hypothesis put forward by Marks and Kammann, but rather confirmed that the target/transcript matches in the first remote-viewing

study are to be attributed (as originally interpreted) to the quality of the subject's descriptions themselves.

Furthermore, in the extensive SRI replication studies³, which also yielded significant results, the Marks–Kammann criticisms do not apply in principle. Target lists and transcripts were separately randomised, and transcripts were carefully checked before judging to ensure absence of any phrasing for which even a weak *post hoc* potential-cue argument could be made.

Given (1) the failure (by empirical test) of the cueing-artefact hypothesis to account for the success of the first-published SRI remote-viewing study², (2) the inapplicability of this hypothesis to the later SRI replication studies³, and (3) the replication of this work in our own and other laboratories, the bulk of which is successful⁷, the data continue to confirm the original conclusion that remote viewing is a viable human perceptual capability.

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Is Cassiopeia A a black hole?

SHKLOVSKY has recently¹ summarised evidence in favour of a black hole in Cas A in contrast to my arguments^{2,3} in favour of a binary neutron star. I defend my view here.

The fact that Cas A has not been reported as a supernova is disturbing as it has an assumed interstellar absorption A_v of 4.3 mag, and a distance of 2.8 kpc. On the other hand, Tammann⁴ has suggested a mean supernova rate of some $10^{-1.1 \pm 0.3} \text{ yr}^{-1}$ for our Galaxy; a similarly high rate is needed to account for the observed neutron stars, both pulsars and transient X-ray sources. These estimates may be reconciled if supernovae are sometimes obscured, by an adjacent dense hydrogen cloud, say, whose absorptivity is

temporarily raised by ionisation. Note that such an explanation would similarly be able to explain the inclination dependence⁴ of supernova rates in external galaxies, and the absence so far of Type 2 explosions in irregular galaxies.

Cas A is anomalously bright at radio frequencies, and one is tempted to conclude that it has an anomalous mass. From optical observations, one infers a mass $<1 M_{\odot}$. If this mass is concentrated in a network of filaments, of electron density $>10^3 \text{ cm}^{-3}$, then the X-ray luminosity can similarly stem from a mass $<1 M_{\odot}$. (McKee's assumption of homogeneous shells need³ not be realistic.) And if the velocity field of the quasi-stationary flocculi reveals³ a peculiar velocity of $(165 \pm 15) \text{ km s}^{-1}$ of the whole remnant, towards north-northeast and towards us, then the supernova remnant must be lighter than its recoil partner (unless the latter moved even faster), again supporting a mass $\leq 1 M_{\odot}$ in the conservative case.

This recoil partner cannot be very luminous because it has not even been detected. Very careful searches have been performed near the divergence centre of the optical knots, whereas the recoil may have removed it southwards. I think that a low mass binary containing a neutron star is a likely candidate. The neutron star's surface temperature⁵ may be anything between 10^5 K and 10^7 K , depending on pion condensation in its interior. Its relativistic wind may be shielded by a cocoon formed from the wind material of its companion. And it can be the source of the cosmic rays in the nebula, and of the soft radio flare⁶ which has probably occurred between 1967 and 1974. Alternatively, the recoil partner may be a second binary pulsar⁷.

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Evidence for late Precambrian plate tectonics in West Africa

BLACK ET AL.¹ have reported further evidence for plate tectonics in the Precambrian based on their study of a 600-Myr-old collision zone proposed^{2,3} along the eastern margin of the West African craton. In places, this margin is associated with a negative-positive gravity anomaly pair⁴. A case for plate tectonics in the Canadian Shield, following the initial suggestion⁵ and a description of the geological evidence⁶, has centred around interpretation of similar anomalies

present at several structural boundaries proposed as collisional suture zones⁷⁻¹⁰. The type structure derived from the anomalies comprises an older crustal block, that thickens towards the suture, in steep contact with a thicker and denser, younger crustal block, both blocks being in approximate isostatic equilibrium¹¹. A similar model has been proposed^{12,13} for the eastern margin of the West African craton. These gravity models (Canadian and West African) are comparable with a 'basement reactivation' model proposed for collided continents¹⁴.

We include the West African anomalies in a growing family of anomalies that seem to be collision-related phenomena reflecting a particular crustal configuration developed at suture zones. The gravity anomalies in the Canadian Shield occur along 1,800 and 1,000 Myr old orogenic belts. The addition of a 600 Myr old example from Africa suggests a continuum throughout most of Proterozoic time (1,800–600 Myr BP). Another example of paired anomalies, currently under study, along the southern Appalachians apparently extends the continuum into the Phanerozoic to ~450 Myr BP. Similar gravity signatures have been described from the Precambrian shields of Australia¹⁵ and India¹⁶. Precambrian collisions proposed in Europe and South America¹⁷ may provide more examples.

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BLACK ET AL. REPLY—We agree with Thomas et al. on the importance and significance of paired gravity anomalies

along collisional suture zones, although we feel that detailed seismic information is required to perfect the model.

Note, however, that the anomalies along the eastern edge of the West African craton fall into two categories: (1) anomalies of long wavelength for which one can envisage a crustal structure similar to that proposed by Thomas et al. to explain coupled negative and positive anomalies along a cryptic suture. In Togo–Benin, however, in assessing the negative anomaly, one has to take into account the sediments of the passive continental margin (Buem, Atacorian). (2) Positive anomalies of shorter wavelength corresponding to more superficial unrooted structures and displaying subvertical to easterly dipping geometry in accord with the general movement pattern with thrusting towards the craton. Field observations show that these anomalies are due to basic and ultrabasic bodies emplaced along the suture^{1,2}.

The location of the two types of anomalies seems to be related to the geometry of the craton, which we believe reflects the original shape of the continent before collision. The first type of paired gravity anomalies occurs in Togo–Benin where the craton forms a promontory and where collision is thought to have been most intense. This situation led to the complete disappearance by subduction of the oceanic floor and of the active margin to the eastern continent, thus bringing into direct contact by underthrusting the low-grade metasediments belonging to the passive continental margin of the craton, and high-grade basement of the eastern continent. In contrast, the second type of anomalies are located in an embayment where island arc and cordilleran volcanoclastic assemblages of the active margin to the eastern continent have been preserved.

The Bouguer anomaly map of Canada suggests that several paired gravity anomalies are arcuate in form and occur along promontories, such as the Thelon front between the Slave and Churchill provinces³.

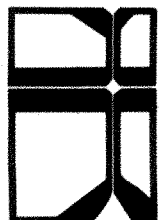
We believe at present that the stress and deformation pattern together with the gravity signature have largely been controlled by the geometry of the colliding passive continental margin^{4,5}.

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BOOK REVIEWS

Essay-writing is difficult

Eric Ashby

"THE essayist," wrote E. B. White, himself a virtuoso in essay-writing, is "sustained by the childish belief that everything he thinks about, everything that happens to him, is of general interest." Essays can be classified as those written to instruct, to persuade, or to entertain. If they have to be limited in length to some 1,200 words, preferably about some scientific topic, they are a difficult art-form for any of these three purposes. This is what Professor Morowitz attempts in this book. It is an assemblage of 57 titbits from the magazine *Hospital Practice*: some to instruct, some to persuade, and all — the author himself confesses — intended to entertain.

To be fair to these essays it is essential to remember that they appeared one at a time, as appetisers, in a magazine devoted (to judge from its title) to the sober topic of how to care for the sick. To offer the reader 57 appetisers, all at once, undiluted by articles on pre-natal clinics and intensive care units, is to expose the appetisers to unreasonable criticism. Only a master in essay-writing could hope to stand up to such criticism. The blurb on the dust-cover "invites comparison to *Lives of a Cell* by Lewis Thomas." This is not fair, for pleasant as some of Morowitz's whimsical pieces are, he is not in the same league as Lewis Thomas, for reasons I shall try to summarise at the end of this review.

Here is a sample of the more enjoyable essays. Morowitz receives a birthday card with the caption: "According to biochemists the materials that make up the human body are only worth 97 cents." He goes through the catalogue of a biochemical company and finds that the market price of the compounds in his body, synthesised from the 97 cents-worth of elements, is of the order of 6 million dollars: a nice reminder that "information is much more expensive than matter." The bicentennial of the United States in 1976 moves him to reflect that 1976 is also the centennial of "the second most significant document produced in the United States, Josiah Willard Gibbs' paper *On the Equilibrium of Heterogeneous Substances*: and there follows an epitome of Gibbs' contribution to science. In another essay he describes a conference of

The Wine of Life, and Other Essays on Societies, Energy and Living Things. By H. J. Morowitz. Pp.265. (St Martin's Press: New York, 1979.) \$10.

ecologists at the Hague and is shocked at the way they fouled the air with cigars distributed at a reception in their honour. This was an unfortunate theme to choose, for it does invite comparison with Thomas' essay on a medical conference at Atlantic City (*On Societies as Organisms*). Lewis flutters like a graceful bird over the scene of the conference; Morowitz (it seems to me) wades through the scene in gumboots. The essayist (if he has the skill) can be Mozartian in 1,200 words; he cannot, in 1,200 words, be Wagnerian.

Occasionally Morowitz does bring it off. He picks on a phrase in a report about experiments on animals: "the animals were sacrificed." This, in Britain, could mean simply that the worker was complying with the requirements of the Cruelty to Animals Act of 1876. But think (writes Morowitz) about the meaning of the word 'sacrifice': "the slaughter of an animal as an offering to God or a deity." He draws a parallel between the practice of animal sacrifice in religions, which is being phased out, and animal sacrifice in the cause of science, which is on the increase: the incinerator replacing the burnt offering, the control group of rats replacing the Old Testament goat which was not slaughtered; the purpose, however, remaining the same: to "ward off disease, illness, and old age . . .". This is excellent satire, and timely for British readers, for there are at present two Bills before Parliament for the reform of the Cruelty to Animals Act of 1876.

The purpose of some of the essays is to debunk, and Morowitz does this well, if a little heavy-handedly. He publishes a nice letter he wrote to his hairdresser, who tried to persuade him to have his hair treated with a conditioner containing nucleic acid (at, presumably, exorbitant cost). So he considers just what effect applications of DNA or RNA might have on the scalp, and he ends by advising his hairdresser to beware of being an agent for the abuse of words like nucleic acid, proteins, lecithins,

and other mumbo-jumbo borrowed from science to promote profits in the cosmetic industry.

One more example: the essay from which Morowitz chose the title for his book. It is as neat an account as one could get into four pages of the way Claude Bernard (whose home was in the Beaujolais region of France) and, after him, Pasteur, were inspired to do some of their best work by considering "how grape juice becomes wine". This is the use of the essay to instruct and it is as instructor that Morowitz is at his best. He is undoubtedly well read, scholarly, and — consistent with E. B. White's prescription — he wants to tell you what has interested him since the last issue of *Hospital Practice*.

Why, then, do I have reservations (not shared by reviewers quoted on the dust-cover) about the quality of these essays? I have two reasons. One is that essays, unlike articles, published lectures, and monographs, have to be judged primarily upon felicity of style. A good essay sparkles with surprises; it captures your attention without being importunate; it can (Dr Johnson said this about essays) be "an irregular, undigested piece" but it has to convey delight, like a kitten playing with a ball of wool. To do this at all is difficult; to do it on a scientific theme is extremely difficult. I don't find Morowitz's style felicitous: a subjective judgement, of course, and maybe it's my fault and not his.

Second, the essay, unlike other kinds of writing, should tell you a lot about the essayist. After reading Montaigne, Lamb, Jorge Borges — and in science T. H. Huxley, J. B. S. Haldane, and Lewis Thomas — you feel you know the essayist: his prejudices, his enthusiasms, what he stands for. The relation between reader and writer has some of the qualities of friendship. Irregular and undigested as essays may be, they add up to the portrait of a distinct personality. This is what I missed in *The Wine of Life*. □

Lord Ashby is Chancellor of Queen's University, Belfast, and a Fellow of Clare College, Cambridge, UK.

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British stratigraphy

F. Wolverson Cope

A Dynamic Stratigraphy of the British Isles. By R. Anderson, P.H. Bridges, M.R. Leeder and B.W. Sellwood. Pp.301. (George Allen and Unwin: Hemel Hempstead, UK, 1979.) Hardback £15; paperback £7.95.

APTLY titled, this book describes the evolution, over a period of about 3,000 million years, of that part of the Earth's crust occupied by the British Isles.

The past two decades have seen a revolution in geological thinking. Major factors in this have been the increasing capability to date rocks radiometrically, the study of palaeomagnetism, remarkable advances in sedimentological interpretation based upon increased knowledge of modern sedimentary processes, the exploration of sea and ocean beds and of the crust beneath them, and the theory of plate tectonics which has arisen from these. This is an appropriate time for some stocktaking.

The authors are to be congratulated on having so successfully re-interpreted, indeed in some cases interpreted for the first time in a meaningful way, the many phases in the history of the British Isles area. They have explained the evolution of this part of the Earth's crust in the light of plate tectonics, and have added considerably to the story by using results of hydrocarbon exploration in the North Sea.

With a necessarily chronological

treatment, the reader will gain a broad appreciation of the stratigraphy of the British Isles and the surrounding seas. The book highlights the value of sedimentological studies in stratigraphy. Fossils are used almost exclusively as ecological indicators; the results of their use in correlation are taken very much as read so that some stratigraphical problems are glossed over.

The account is well written. It is profusely illustrated with carefully executed maps and diagrams, and the volume is pleasant and easy to use. Though not advocating expansion in technical vocabulary, one feels that a tautological expression such as "facing confrontation" (page 165) should be eliminated. On the other hand, technical terms which come so readily to hand are sometimes better avoided as in "Perhaps palaeoslopes were directed southwards in this area" (page 234) when speaking of slopes.

This book should be invaluable to students and others who wish to gain some understanding of the evolution of the Earth's crust in the British Isles area. It should serve many generations as a useful framework of spatial and dynamic relationships without which stratigraphy must fail to inspire.

Adopting a metaphor suggested by non-use of the adjective *seminarial* (in the gloss on the back of the book), one would say that the four authors have combined most successfully in the production of a lively 'zygote', especially as it might never have come about but for "... a seminal [sic] encounter"! □

F. Wolverson Cope is Professor Emeritus in the University of Keele, UK.

Chinese revelation

M. Christine King

China's Road to Development. Edited by Neville Maxwell. Pp.365. (Pergamon International Library: Oxford, 1979.) Hardback £21; paperback £5.25.

It is a strange fact of life that those most responsible for making history have always been the least able to record it for posterity. The result of this is that, from time to time, history has to be re-written. The present volume on China, an enlarged edition of a collection of essays first published in 1976, is perhaps an apposite example of this phenomenon. It appears at a time when China is deliberately revealing its own past in order to point a way to the future. Thus many of the 'development' theories outlined in the work already form part of history, a past which must now be re-examined. Few are more aware of this than the editor, who has the courage to admit in

his prefatory note that "the papers in this collection have to some extent been outdated".

The book consists of 16 essays by different authors covering developments in China since 1949 as seen in its entirety from the western point of view. Between them the authors cover rural development, industrialisation, agriculture, health care and population control, economic planning and trade, environmental protection, women's role in the country's development, forestry, intermediate energy technology, Chinese foreign aid, city planning and the role of the People's Liberation Army. Curiously, the place of science, the very foundation on which China's new history will be written, is relegated to a passing comment.

Often proffered as the ideal model for Third World development, China's growth has provided a burgeoning of material for commentators — so much so that it is often difficult to discern which of the opinions are really derived from Chinese experience and which are merely the theories of development experts. Thus, in a sentence

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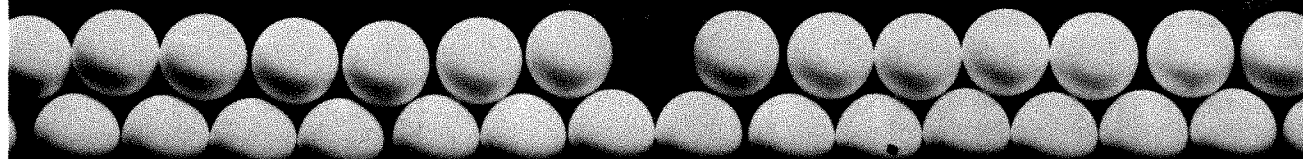
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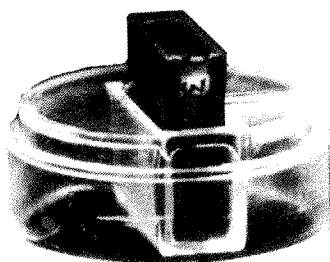
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such as "Although the domination of science by politics during both these periods had an adverse effect on scientific development and on the economy in general, there was also a growing body of opinion that these massive campaigns were a necessary pre-requisite to generating political will for Mao-style development" (page 219), it becomes difficult to know which growing body of opinion is being considered.

Nevertheless, considerable printing errors apart, this volume of essays will remain a valuable source, as much a reflection of western social ideals in the 1970s, as of China's development. Not unexpectedly, for a collective work by different authors, written at different times and clearly aimed at different levels (a few no doubt originally intended as University lectures), there are repetitions of facts and discontinuities in style which detract from the overall impression of the book. But it does raise pertinent questions in the light of China's present changing policies: can similar development goals be achieved by roads other than that of revolution? The answer today may be very different from

that which seemed obvious not so very long ago.

The re-writing of any chapter of history, particularly when done at a distance, calls for no small amount of self-assessment. Leo Orleans, long respected as an analyst of Chinese affairs, expresses the new western dilemma: "It is possible to feel some regret that her [China's] drastic veering did not settle on some more middle course. Mao's dedication to the creation of an egalitarian society, his "serve the people" ethic, the sense of idealism he attempted to instill in the people, captured imaginations outside China as well as within, and some of those who disagreed with China's politics and policies could still identify with Mao's utopian ideals, [which] set China apart from every other nation" (page 225). By choosing a new and different road to development, China has set not only new goals for itself, but also a whole set of fresh criteria for western analysts. □

M. Christine King is researching into Chinese Affairs and the History and Philosophy of Science.

Higher marine fungi

R. A. Eaton

Marine Mycology: The Higher Fungi. By J. Kohlmeyer and E. Kohlmeyer. Pp. 690. (Academic: New York and London, 1979.) \$59.50; £34.60.

FOR 20 years, the authors' research in the field of marine mycology has produced a high quality of illustration and description of marine fungi, particularly through their *Icones Fungorum Maris* and *Synoptic Plates of Higher Marine Fungi*. A large part of this volume combines the essence of these publications to give us the most comprehensive work since Johnson and Sparrows (*Fungi in Oceans and Estuaries*, J. Cramer: Lehre, FRG, 1961) that any student of marine fungi would need.

More than half of the book comprises illustrated keys, and descriptions of higher marine fungi including 90 plates of micrographs. But is photographic representation the best means of illustration? Certainly the beautifully accurate line drawings in the *Icones* are not surpassed by many micrographs in this book. Their use of interference contrast microscopy introduces a new dimension for observing the appendaged ascospores of many marine fungi, but has its limitations in aiding interpretation of sectioned ascocarps or spermogonia, and non-appendaged spores.

Parasitic and saprobic marine fungi exist on various natural and man-made substrates from mangrove vegetation to polyurethane. They are distributed in different geographical zones and at different depths in the oceans. The book deals very comprehensively with the factors which affect the occurrence of filamentous fungi and yeasts in the sea, but the authors consider only a "selection of pertinent papers" on their physiology and biochemistry.

Other chapters are concerned with methodology, fruit body and spore ontogeny, and the release, dispersal and settlement of spores. The fascinating relationships which exist between fungi and marine animals (particularly marine wood borers) and between fungi and algae (producing marine lichens), are also covered. Apart from a chapter on marine yeasts this book is concerned wholly with filamentous higher fungi, as the title states. For the sake of completeness, was it not possible to broaden the scope of the book a little, and include more than a mention of the lower marine fungi — one-fifth of the total marine mycota?

The authors state that "a major objective of this treatise is to assist students of marine fungi in the identification of species." This is achieved, for it is unquestionably a reference book to be kept on the shelf above the microscope. □

R.A. Eaton is Senior Lecturer in the Department of Biological Sciences, Portsmouth Polytechnic, UK.

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OBITUARY

O.R. Frisch, 1904-1979

ON 22 September 1979 Professor Otto Robert Frisch, OBE, FRS, Emeritus Jacksonian Professor in the University of Cambridge, died following an accident. He was a distinguished and original nuclear physicist. His contributions to physics will be, and the example he set in his approach to the subject should be, remembered by future physicists.

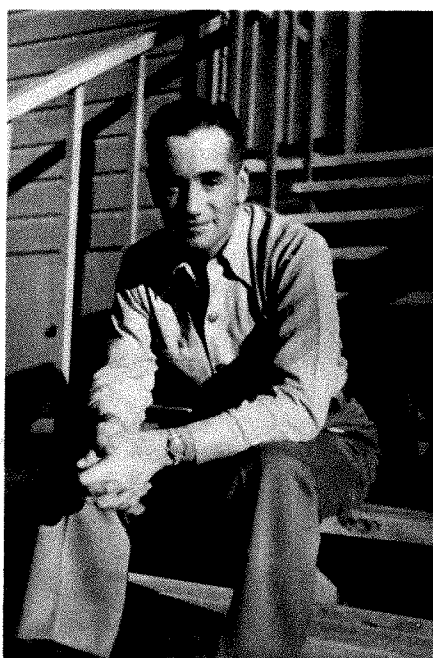
Both in physics and in music, which was next to physics his strongest interest, his main driving force was enjoyment of what he was doing. In physics this meant using simple apparatus, preferably designed and made by himself, and looking for important problems with transparent answers. Some of the instruments he made might be called gadgets, but surprisingly often these gadgets were able to provide answers to topical problems. Naturally "big physics" was not his line, and he never became involved with projects needing large machines and large teams of collaborators.

His piano playing was of near-professional standard, but he was not a perfectionist, and was not above listening to, or playing with, musicians of a much lower calibre, provided they shared his enjoyment of the result.

He was born on 1 October 1904 in Vienna. His father, Justinian Frisch, was a printer, his mother a very gifted musician. I do not know whether his father's profession influenced his interest in writing and his flair for the written word, but no doubt his mother encouraged his music. The members of the family were very close, and all his life he remained in touch with his relatives. Above all he was very close to his aunt, Lise Meitner, the famous nuclear physicist.

At school Otto Robert (as he was known to the family; later he was just Robert to his friends, except for the Los Alamos period, when there were too many Roberts around, and he became Otto) showed marked ability in mathematics, and almost chose it as a career, but in the end decided that this would be too abstract for his taste, and he settled for physics.

His studies in the University of Vienna under Professor Karl Przibram appear to have been uneventful, and he obtained his D Phil in 1926, after four years in the university. This was rapid progress, but at the time not unusual. After a year in a small firm run by an inventor, where he presumably could indulge in his love for gadgets, he accepted a post in the Physikalisch-Technische Reichsanstalt in Berlin. His work there was in the optics division, with the assignment to work on a proposed new light standard, but he says "I stayed long hours trying out half-baked



ideas of my own". His assignment was hardly exciting, but he was able to attend seminar meetings at the university, and listen to remarks by Einstein, Planck, Nernst and others. Lise Meitner was there, too. She worked, and lived, in the suburb of Dahlem, but Frisch saw her frequently, to the profit of his physics and his music.

In 1930 he became an assistant to Otto Stern in Hamburg, famous for the Stern-Gerlach experiment. Stern was an outstanding experimenter, not in manual skill, but in his command of the relevant principles and in the design of experiments, and his laboratory was the leading one in the world for atomic-beam research. Frisch worked closely with him and took part in important experiments, including the first observation of the diffraction of atomic beams by crystal surfaces. At the end of his Hamburg period he did his first important experiment on his own, observing the recoil of atoms on photon emission.

But by this time the Nazi government was in power, and both Stern and Frisch, who were of Jewish descent, had to leave. Frisch went to Birkbeck College to work under Blackett with a grant from the Academic Assistance Council. Here he became interested in nuclear physics, which in 1933 was full of excitement. He invented a method of measuring very short-lived activities, and this led to the discovery of some new radioactive isotopes.

A year later, Niels Bohr, who must have heard of his ingenuity in devising and carrying out experiments, invited him to Copenhagen. The next five years in the informal and stimulating atmosphere of

Bohr's Institute were very fruitful. It was the time when neutron physics had been initiated by Fermi's work, and Frisch, too, started on neutron work. Probably he was drawn in initially because he happened to have the most appropriate counter with which to test for neutron-induced activity. He made many important contributions to that subject.

It was in neutron physics that he did his most famous piece of work. He was spending Christmas 1938 with Lise Meitner, who was now in Sweden. She received a letter from Otto Hahn, with whom she had collaborated closely until she had to leave Germany, telling her of his discovery that barium was among the products emitted when uranium was bombarded with neutrons. Hahn realised this meant that the uranium nucleus must have split into two large pieces. Frisch and Lise Meitner were as dumbfounded by this news as all other physicists when they heard of it. But after thinking about the discovery for a while, they understood how the strong electric charge could help to overcome the surface tension and lead to an unstable configuration once the nucleus was set vibrating by a collision. If the nucleus had taken a sufficiently elongated form the mutual repulsion of the positive charges would drive it apart, and this would release an energy of about 200 MeV. Frisch coined the word "fission" for this process. After returning to Copenhagen and composing a letter to *Nature* jointly with Lise Meitner over the telephone to Stockholm, he carried out an impressively simple experiment proving the emission of energetic and heavily ionizing particles from the bombarded uranium, and thus confirmed the interpretation.

Fission was now the exciting part of nuclear physics, and most nuclear physics laboratories started to work on this. Frisch continued in this field, too, and did several more important experiments.

But this was 1939. War was imminent, and Denmark not too healthy a place for a German refugee. (The Anschluss had made him a German subject). Besides, the Danish police were making difficulties about extending his permit. So he tried to find a place in England, and, when Oliphant heard of his problem, he arranged an appointment for him in the University of Birmingham. Here Oliphant was trying to build up facilities for nuclear physics work, but there was not much equipment as yet, and Frisch could not continue his experimental work immediately. But he continued speculating about fission.

The possibility of the release of nuclear energy was then in many people's minds,

once it had been shown that neutrons were emitted after the fission. One was awed by the thought of a new weapon of terrifying power, but Frisch, like others who had understood the paper by Bohr and Wheeler on the mechanism of fission, and Bohr's argument that the fission caused by slow neutrons was due to the rare isotope ^{235}U , were reassured that no violent explosion could happen in ordinary uranium. The thought of separating uranium isotopes in large quantities seemed to belong to science fiction. Then one day Frisch came to me and said "Suppose one had a large amount of separated ^{235}U , what would happen?" One could make a guess at the fission cross section from the ideas of Bohr, and I knew how to estimate the critical size, given the cross section. The answer staggered us by being smaller than we would have guessed. Next we had to estimate how far the chain reaction would proceed before the energy it released would drive the uranium apart. The answer again staggered us by being a reasonable fraction of the total energy available. If that was so, we said, it would be, as a weapon, worth its price, even if the isotope separation plant cost as much as a battleship (this turned out to be an underestimate). What if the Germans got there first and a nuclear bomb was in the hands of Hitler's Germany?

We wrote down our arguments, and that was the start of serious interest in atomic energy work in England. Frisch first tried to explore the possibility of isotope separation by thermal diffusion, but did not get far, and we now know that the thermal diffusion coefficient in the only gaseous uranium compound happens to be practically zero. Instead Frisch moved to Liverpool, where in Chadwick's laboratory there was a cyclotron and other facilities for nuclear physics, and worked on the nuclear physics aspects of atomic energy until the end of 1943, when it was decided to discontinue work in England and move to America those who could be useful to the American work.

So Frisch went to Los Alamos, the strange atom-bomb city in the middle of New Mexico. He was not attached to any specialist team but had a roving assignment and in that way was able to give essential help to many different groups. One of the experiments he set up himself was a typically original scheme known as the dragon. Two pieces of fissile material which together would make a critical mass and so cause a violent chain reaction, were allowed near each other for only a short moment, by dropping one piece past the other. The speed was so arranged that the chain reaction did not develop to any dangerous degree. The name indicated that one was tickling the tail of a dragon. The experiment made it possible to observe details of a near-critical situation which were otherwise hard to get at.

After the war, Frisch returned to England and became head of the Nuclear Physics Division at Harwell. Adminis-

tration was not his favourite pastime, but he found that his deputy Dr (later Sir) Robert Cockburn delighted in running things and did it well. Frisch left Harwell in 1947 to become the Jacksonian Professor of Physics in Cambridge and a Fellow of Trinity. At that time nuclear physics in Cambridge was overshadowed by crystallography and other lines. It was not in Frisch's character to fight for more support. Besides, nuclear physics had now become big physics and less to his taste. While he kept a lively interest in nuclear physics and in the newly developing particle physics, he spent more of his time teaching and writing. Many students profited from contact with his way of doing and looking at things. He still liked gadgets, and made many ingenious instruments. His writing included *Meet the Atoms*, a very readable popular introduction to modern physics. Only a few months ago he published his recollections under the appealing title *What little I remember*,¹ and it is pleasing that he was able to leave this lively record of his personality.

Shortly after moving to Cambridge he married, and there were two children. His wife shared not only his Austrian origin, but very many of his attitudes and his love for music.

Shortly before retiring in 1972 he invented an apparatus for measuring and evaluating bubble-chamber tracks, and he later became a partner in a firm set up to manufacture this. I do not know whether this commercial activity made him a wealthy man, but he clearly enjoyed this novel position and seeing his gadget being produced.

His was a full life, but he just failed to reach his seventy-fifth birthday with its warm messages from his many friends.

Rudolf Peierls

1. Frisch, O.R. *What Little I Remember*. (Cambridge University Press, 1979) See review by Rudolf Peierls in *Nature* 280, 257-259 (19 July 1979).

Martin Lüscher

IN THE midst of his fruitful work, Martin Lüscher's life was taken on 9 August 1979 by a tragic accident, at the age of 63. He was Professor of Zoology at the University of Berne, and directed its Department of Zoophysiology with scientific foresight and a fatherly concern for his colleagues and students. His name will be remembered by zoologists throughout the world.

Martin Lüscher, son of the Basle artist Jean-Jacques Lüscher, spent a quiet youth in his family home, with extended visits to Provence. There, the harmony and beauty of the landscape created a lasting impression on him; in the balmy

Mediterranean atmosphere with the colour play and diverse insect cacophony in the flower fields he developed a love and interest of plants and animals. In Basel in 1944 he was awarded a PhD in zoology. After his marriage to Noemi Stoecklin, daughter of the Basle artist Niklaus Stoecklin, which proved to be a true life partnership, he began, with her, his life-long progress as a zoologist. They shared a life of work, pleasures and trials. A great deal of his professional success was due to his close accord with his wife, with guests always welcome at their home.

As a young zoologist, Lüscher's first post was as a research assistant in Berne, where he worked under F.E. Lehmann on the physiology of development of amphibians. Soon afterwards he met his great teacher, the insect physiologist, Sir Vincent Wigglesworth of Cambridge, under whom he was able to work. Martin Lüscher saw Wigglesworth as the founder of a new branch of research: experimental insect physiology.

From England Lüscher moved to Paris, where he was introduced to the biology of termites by the renowned termite specialist T.P. Grassé and found the stimulus for what was to become his principal research — caste formation in termite colonies. He wished to study termites in the field as well as the laboratory, and he soon had the opportunity to take part in an expedition to observe at first hand the highly organised termite colonies of Africa. Lüscher was particularly fascinated by the highly elaborate nest structures built by the millions of termites within the colony. He was the first to understand their design as a well planned respiratory system with temperature and humidity regulation, and an air circulation system functioning through the warming of the air inside the nest. After imaginative and productive field work he spent a very important year of study in the United States on a grant from the Rockefeller Foundation. He was given the opportunity to stay for some time with A.E. Emerson, the expert on termite biology and taxonomy, and was then able to acquaint himself with modern methods of insect endocrinology in the leading laboratories at Berkeley, and at Harvard with C.M. Williams. Afterwards he worked at the Swiss Tropical Institute in Basel and in 1954 was appointed professor at the University of Berne. Under his direction the new Department of Zoophysiology was formed.

In 1965 he was appointed to the four-year directorship of the Zoological Institute. During the academic year 1967/68 he was Dean of the natural science faculty and from 1969 a member of the Swiss National Science Foundation. In his last important undertaking, in Nairobi as project leader at the newly founded International Centre of Insect Physiology and Ecology, he set up an enthusiastic research team.

Lüscher carried his title with quiet

modesty. His lectures were simple and clear, and his criticism was always constructive and helpful. His motivation as a researcher derived from the pleasure of observation, an urge for knowledge and a keen understanding of relationships. He was intrigued, like many earlier researchers, by the secretive world of the termite colony. How do all the different castes including the workers, the soldiers and the reproductive individuals develop from the same origin in such numbers? How is this ratio maintained and regulated? His understanding of the termite colony as one organism, which he had gained in Africa, and the methods of experimental physiology he had learned, led to further discoveries. He found that all the castes of the termite colony interact continuously, as though they were parts of a single organism. The presence of reproductives in lower termite colonies inhibits the development of that caste, whereas their absence induces the formation of further reproductives. In a similar way the ratio of developing soldiers is regulated by the proportion of soldiers present in the colony.

Lüscher became aware of the importance of 'social hormones' as agents of regulation and communication within the termite colony, and with the biochemist Peter Karlson he introduced the now well-known term pheromone. He could demonstrate by means of ingeniously simple experiments that pheromones are released from the anus of the reproductive individual and are ingested orally by other termites and then distributed throughout the colony by oral exchange, the so-called process of trophylaxis. Pheromones regulate the make up of the social structure, whereas hormones determine the process and direction of development of the single individual, and the latter are controlled by the former. It was established experimentally that the inhibition of reproductive caste development could be simulated by application of 'juvenile hormone', which in general inhibits the metamorphosis of larval insects into adults. The same hormone applied in higher doses to the termite society was shown to stimulate soldier production. With these findings, Lüscher made a breakthrough towards a new physiological understanding of termite caste regulation. The antagonistic effect of reproductives and soldiers that inhibit the development of their own caste and open the way towards development of the other could be interpreted physiologically: the reproductive castes transmit, by means of their pheromones, information causing the developing larvae to produce their own juvenile hormone, whereas the soldiers, probably also by means of specific pheromones, transmit information to inhibit juvenile hormone.

Recent results from Lüscher's team in Nairobi and Bern suggest similar exocrine-endocrine principles of caste regulation for

the highly organized mound-building 'higher termites.'

To understand all these interrelationships Lüscher augmented his work with basic research into the mechanism of hormonal interaction. He investigated the mechanisms of action of juvenile hormone and the ecdysteroid moulting hormones. He studied the interactions and regulatory mechanisms of those hormones in the cockroach, a nonsocial insect related to termites.

Lüscher's name as a termite researcher and insect physiologist has international acclaim. He retained, however, his love for the countryside, for plant and animal life and for his fellow men. I best remember the hours I spent with him in the African bush, as we stood looking with astonishment at an opened termite mound. Here lay the motivation and drive for his life's work: the questions arising from his awe and respect at the incomprehensible and beautiful. This ethos together with scientific accuracy and genius gave him and his work its far reaching importance.

Reinhard Leuthold

G.S. Adair

GILBERT SMITHSON Adair, who died on 20 June 1979, was a highly respected and well loved character on the Cambridge scene for sixty years. Many generations of students and research workers have cause to be grateful for his willing help with practical advice and theoretical explanations. This ranged from supplying Max Perutz with his first haemoglobin crystals and instructing people in the art of preparing collodion membranes of varying permeabilities, to simple explanations of Gibbsian thermodynamics. In this way he exerted considerable influence although he was, to the best of my knowledge, only formally responsible for the supervision of two research students; Muriel Robinson (who became Muriel Adair) and the author of this notice.

Adair was born on 21 September 1896, the son of a Quaker family. He went up to King's, Cambridge, in 1915 and remained in that city, with only very brief interruptions until his death. He was a Fellow of his college from 1923 to 1933 and an honorary Fellow after retirement from his University post (Reader in Biophysics) in 1963.

The wide interest of biophysicists in haemoglobin, which extended over the last 60 years, involved several distinguished Cambridge men. Adair had the stimulation of A.V. Hill, Barcroft and Roughton, who not only contributed to our knowledge about the respiratory function of haemoglobin, but also used this important

and plentiful protein to develop novel thermodynamic and kinetic methods. The iron contents had given an equivalent weight for haemoglobin of about 16,700 Daltons. Brown and Hill had shown that comparison of calorimetric and van't Hoff ΔH for oxygen binding gave about 17,000g of haemoglobin per mol of binding site. Osmotic pressure measurements preceding those of Adair gave variable results and tended to support the prevailing theory that haemoglobin occurred in solution with a molecular weight of about 17,000 and that there was some tendency towards aggregating of these units. Adair's attention to experimental detail is illustrated in a masterly paper "A critical study of the direct method of measuring the osmotic pressure of haemoglobin" (*Proc. Roy. Soc. A*108, 627, 1924) in which he reports a molecular weight of 67,000 and explains the erroneous results of others. It is important to point out that this preceded by about a year, Svedberg's determination of the molecular weight of haemoglobin in the ultracentrifuge.

Further studies of the osmotic pressure of haemoglobin solutions led Adair to detailed experimental and theoretical investigations of membrane potentials. In the 1930s he extended these studies to serum proteins and to the determination of charges on protein molecules from the measured membrane potential at different pH and ionic strength.

The above contributions led to his election to the Royal Society in 1939. During the war Adair was much occupied with the teaching of physiology, especially in practical classes. After the war, one of his main concerns was to improve his simple technique for measuring osmotic pressures, so that small pressures of solutions of large protein molecules could be measured. He also found it difficult to believe the results of his second research student, which indicated reversible dissociation of haemoglobin molecules in dilute solutions and at high ionic strength, until he confirmed it himself with his refined methods.

Adair was a shy but very friendly man who was absolutely devoted to his rather specialised, but very valuable work in the laboratory. The essence of his experimental technique was simplicity and attention to detail. Old fashioned alarm clocks and domestic heaters formed the backbone of his wonderful method for making semi-permeable membranes.

Apart from his scientific work Adair read widely and, in his youth, was a keen rock climber. There are a number of stories about his climbing activities at college and into the Physiology Laboratory. From 1931 until her death in 1977, he shared a life of work and simple pleasures with Muriel Adair. Their house near Grantchester Meadows allowed him to pursue his interests in the observation of a variety of plant and animal life.

H. Gutfreund

20 March 1980

Using the scientific expertise of the House of Lords

THE idea for a House of Lords select committee on science and technology came from Lords Shackleton and Sherfield last year — shortly after the reorganisation of the House of Commons select committee structure. This reorganisation has dealt a severe blow to the interests of science and science policy in Parliament because each select committee covers the remit of a single government department. Science now comes primarily under the select committee for education and science — instead of the science and technology committee of the old structure — although topics of scientific content may also touch on the interests of other committees such as agriculture or energy.

But the education and science select committee is likely to spend more time on education than scientific matters, and none of the other departments is likely to provide a proper forum for scientific discussion of issues which embrace many departments — like biotechnology, for example. Lords Sherfield and Shackleton proposed a select committee in the House of Lords to fill the gap. Parliament and government were very badly informed on science political matters, they felt, and select committees were an invaluable way of gathering information. A House of Lords committee would also have the advantage that its members could be drawn from the many respected scientists and engineers who sit in the Lords. It should therefore be able to bring more scientific expertise to bear on key issues of science policy than its predecessor in the Commons.

The first indication of its intentions came last week, when the new committee — the 'House of Lords Select Committee on Science and Technology' decided on the first two subjects for its inquiries: forestry and electric vehicles. Two subcommittees are to be set up to consider them: one, under the chairmanship of Lord Sherfield, will look at the role of fundamental research in relation to British forests and woodlands; the other, under the chairmanship of Lord Gregson, will review the case for electric vehicles in the light of present energy shortages.

These subjects are intended to fulfil the criteria laid down for topics of inquiry when the select committee was first agreed: that is they should be of interdepartmental interest, and therefore fall between the interests of several Commons select committees, or they should be of insufficient relevance to any government department to be considered at all.

Forestry is a subject which might easily have come under the Ministry of Agriculture, Fisheries and Food or the Department of the Environment. The House of Lords committee hopes to be able to ask questions which will be relevant to both departments and introduce an interdiscip-

linary approach.

Electric vehicles, on the other hand, might have come under the Department of Energy, but with the seemingly weightier problems of nuclear energy to consider, the House of Commons energy select committee would be unlikely to get round to the subject for a long time. The House of Lords committee may well, in this case, be looking at a worthy topic which might otherwise have been neglected — at least until its topicality had been lost.

Neither of these subjects, however, fires the greatest enthusiasm. They are hardly the most pressing of current issues of science and technology policy. Nevertheless they are both intended to be fairly short investigations: in particular, Lord Gregson's subcommittee on electric vehicles is due to start taking evidence shortly after Easter and to report in the autumn. Further plans are still tentative, although the committee has earmarked "aspects of information technology" and the role of science policy making bodies and their relationship to government as its next subjects for investigation. Specific topics within these broad subjects will have to be identified if useful inquiries are to be made. Under the second subject, for example, a look at the role of the National Research Development Corporation or the Department of Industry's requirements boards could be useful.

However, the committee should not shy away from considering highly political topics. Last week Lord Shackleton said that the committee had been asked "to avoid questions where the political component is much larger than the scientific". But the greater the political overtones of a scientific issue, the more important it is that it should be investigated by a select committee with scientific expertise.

Lord Shackleton also said that the committee would take an interest in "things that haven't been taken into account elsewhere". This could be incisive, if topics are chosen wisely; but it could also be a recipe for bumbledom, particularly if the committee allows itself to be steered away from the politically hot scientific topics.

One possibility that seems to have been ignored is that the committee should use its scientific expertise, and its ability to appoint scientific advisors, directly: why not look in technical depth at scientific issues which are in the province of the Commons committees, but cannot be covered there at sufficient length? Take the question raised by Sir Alan Cottrell recently about the feasibility of guarding pressurised water reactor pressure vessels against dangerous cracks. This is a fine scientific and serious political issue to which the Lords committee could pay profitable attention. □

United States

Gasohol enthusiasm may increase world food problem

A warning that growing enthusiasm for the use of agricultural crops to produce automobile fuels may contribute directly to escalating food costs and shortages has come from Lester Brown, Director of the Washington-based Worldwatch Institute.

In a report financed by the United Nations Environment Programme and the Gund Foundation, published last week, Mr Brown draws attention to potential conflicts between the different users of such crops, arguing that the demand by motorists for fuel produced in this way represents a major new variable in discussions of food supply and population growth.

"The stage is set for direct competition between the affluent minority who own the world's 315 million automobiles, and the poorest segments of humanity, for whom getting enough food to stay alive is already a struggle" Mr Brown says in his report.

The rapidly rising cost of petroleum has made the use of alcohol as a fuel — either in pure form, or mixed with petrol to produce gasohol — increasingly attractive, in addition to its environmental advantages and the fact that its production can provide an important source of jobs in developing countries.

The most advanced gasohol programme is in Brazil, where according to Mr Brown the government has set a target of producing 10.7 billion litres of alcohol by 1985. The US also has an ambitious programme, with grants and tax credits designed to reach a domestic production level of 2 billion gallons of ethanol by the mid-80s.

Plans for developing major programmes are also under way in other food exporting countries such as Australia, New Zealand

and South Africa. The consequence, says Mr Brown, is that the possibility exists for the first time of a major shift of food production capacity to non-food purposes".

Yields can be high, and could be boosted even further by a "modest effort in plant breeding and research" he suggests. As a result, the economic conditions for the large-scale commercial production from high-yielding energy crops appears to be favourable.

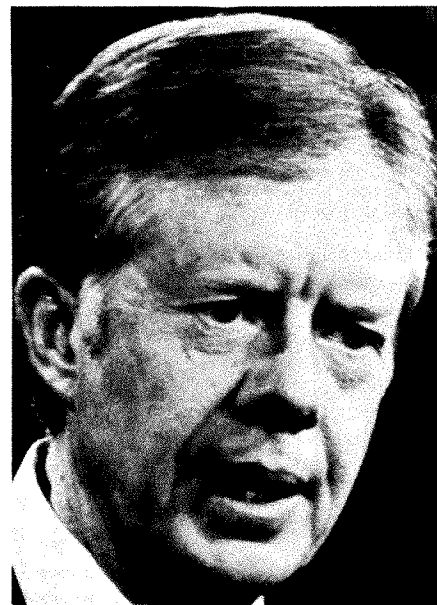
But the impact on food supplies and prices could be severe, particularly at a time when increases in world food output are scarcely keeping up with population growth. In Brazil, for example which already has a net deficit of 6 million metric tons a year, meeting official targets could mean planting one fifth of the country's cropland with sugarcane, driving food prices upward and "leading to more severe malnutrition among the poor".

Similarly the US, currently the world's largest supplier of grain, would have to reduce its exportable surplus considerably to meet official production goals, as distillers take a growing share of the US grain harvest. Mr Brown estimates that an individual in the US running a car solely on alcohol would require almost ten times the cropland needed to produce food for an affluent diet. Even using only a 10% ethanol mix, the car owner's cropland requirement would double.

But the solution is not to abandon gasohol. "A carefully designed alcohol fuel programme that gave farmers a first priority in the use of ethanol for tractors, farm trucks, and irrigation pumps would be a major step toward the creation of a sustainable food production system, and of a sustainable society", Mr Brown says, followed over the longer term by a programme based on forest products and cellulosic materials of forest origin. □

Sugarcane: bad-news fuel crop?

'Food or fuel: new consumption for the world's cropland' Worldwatch Paper no 35 Worldwatch Institute, 1776 Massachusetts Avenue NW, Washington DC 20036. \$2.



Carter: synfuels strategist

Controversy over \$20 billion synfuels programme

AFTER three months of intense negotiation, the US Congress is putting the finishing touches to legislation which will commit the government to spending \$20 billion over the next four years to stimulate the production of synthetic fuels. But the proposed legislation remains surrounded by controversy, in particular over the extent to which political interests have been allowed to override environmental and technical concerns.

The synfuels programme, a major plank in President Carter's strategy to reduce dependence on imported oil, would provide a variety of financial incentives to encourage private industry to construct plants producing synthetic oil or gas from coal, oil shale or other sources.

The proposal has been eagerly pushed by a number of constituencies, such as coal companies and engineering firms, who see it as a potential source of generous government support.

But it still faces a battery of critics, from environmentalists who argue that the health and environmental implications of a crash synfuels programme are receiving inadequate attention, to some parts of the oil industry which feel that a massive federal investment, based primarily on existing technology, could seriously distort the future growth of the industry.

So far the environmentalists have received little comfort. They have raised numerous concerns, such as the large quantities of water needed by synfuel plants, and the potential carcinogenic

hazards arising from the emission of aromatic and hydrocarbon amines. But legislators have indicated that they are more concerned about producing energy than meeting social and environmental costs — and are unlikely to produce much extra money for research into the latter.

Neither has much been done to calm the fears that a programme on the scale envisaged by the administration — initially aimed at producing 500,000 barrels a day by 1985 and 2 million by 1990 — is overambitious, and likely to encounter problems such as an inadequate technology base and a severe shortage of appropriately qualified scientists and engineers.

Typical reservations were expressed at a two-day meeting organised last October by the National Academy of Sciences, and attended by a cross-section of industrial and academic scientists and engineers. This reached the conclusion that the 1985 deadline could not be met, but would require six to eight years to achieve.

The meeting also pointed out that reaching the 1990 target would require second stage technologies to be selected before the results of the first stage had been properly analysed.

Following these and other similar

criticisms, the administration's initial proposals have been tempered. Thus the President has agreed that the initial cost of \$88 billion be broken down into two stages, with Congress committing itself only to \$20 billion in the first four years.

Following widespread opposition from the oil industry over what it considered to be excessive federal involvement and unfair competition, the president has also agreed that the synthetic fuels corporation which will be responsible for the programme will not have the authority to construct and run its own plants, but merely assist the private sector to do so.

Furthermore members of the Senate and the House of Representatives, meeting last week to resolve differences in their respective versions of the synfuels legislation, agreed that the deadlines for the 500,000 and 2 million barrels a day targets be set back to 1987 and 1992 respectively.

But the major thrust of the administration's strategy of pushing for ambitious production goals remains in place. These goals had been strongly supported by the Senate, but opposed by the House which had proposed a more modest package — costing only \$3 billion and with no new corporation — and

suggested a greater emphasis on conservation measures to meet the same goals.

As far as manpower needs are concerned, Dr Alex K. Logwunik of Pullman Kellogg, one of the largest engineering firms in the country, estimated last month that the proposed synfuels programme could absorb 20% to 60% of the capacity of the ten major engineering firms in the country. And he warned that the result could be a series of shortages of qualified personnel, already beginning to affect other areas of energy technology.

Supporters of the synfuels programme, however, argue that similar claims had been expressed before other large-scale national efforts, such as the Apollo moon landings. And that once the programme is under way, it will stimulate a flow of research workers and technical people from other areas.

Critics are unconvinced. But in an election year both Congress and the White House are keen to be seen to be doing something about the energy problem — and responding to the frustration of constituents takes precedence over the finer logic of technological timeliness.

David Dickson

Split over nuclear export licensing

FACED with growing difficulties in implementing its non-proliferation policies, the Carter administration is seeking to simplify its task by removing an independent check on the adequacy of safeguards covering nuclear export currently provided by the Nuclear Regulatory Commission.

The NRC itself is split over whether such a move is desirable, but it is being strongly resisted in Congress, for in passing the Nuclear Non Proliferation Act of 1978, legislators specifically invoked the NRC to try to prevent the non-proliferation debate from becoming too politicised by remaining within the sphere of the State Department and the White House.

Under the terms of the Act, the NRC is required to evaluate whether facilities to which nuclear fuel or technology is being exported contain sufficient technical and institutional safeguards to ensure that neither could be diverted to military purposes. However its decision to reject a license application can be overturned by the President — whose decision can in turn be reversed by Congress.

In recent weeks, the White House has been faced with a number of sensitive decisions on nuclear exports. Some in particular have been the direct result of the two-year time limit imposed when the Nuclear Non-Proliferation Act was signed in 1978, requiring nuclear shipments to be cut off after 10 March 1980 to all countries which had not renegotiated nuclear agreements along the lines of the Act.

Attempts to enforce controls on the export of enriched uranium to Europe,

however, were bitterly resisted by leaders of the European Economic Community in the summer of 1978, particularly because of the restrictions placed on the transfer of fuel originating in the US.

With the US administration's failure to generate a consensus behind its general strategy at the International Nuclear Fuel Cycle Evaluation (INFCE) meeting in Vienna last month, it is reluctant to provide further embarrassment.

So, last week President Carter agreed that, rather than bring an embargo into effect, western nations would be given permission to continue buying American enriched uranium as reactor fuels, even though they have not complied with the terms of the 1978 Act. The dispensation is allowed for one year, under the President's power to allow exemptions while negotiations are taking place.

A more difficult case is raised by India, which has persistently refused to sign the Nuclear Non-Proliferation Treaty or to accept international inspection of its nuclear facilities — two requirements for being granted export licenses under the 1978 Act — as being unwarranted interference in internal affairs.

The Administration is being pushed by Congress and environmental groups to maintain a firm line in refusing to grant export licenses covering a shipment of 21 tons of uranium for nuclear power plants at Tarapur in India. Under the terms of the Act, nuclear shipments are now banned unless the President determines otherwise.

However, President Carter is thought to

be actively seeking ways of not offending Mrs Gandhi, indicating last week that he is now merely seeking assurances that the fuel will not be used for explosives, rather than any broader commitment — a decision that would probably be accepted by Congress.

In a separate domain, the US is actively engaged in trying to persuade Switzerland not to export to Argentina a facility capable of producing heavy water, which the administration feels could, as in India several years ago, be used to help produce weapons grade plutonium.

Officials in Washington are said to be worried not only about the fact that this would make it easier for Argentina to construct its own nuclear weapons, but also about what they see as a lax attitude by the Swiss authorities in demanding looser safeguards on nuclear exports than had been agreed in 1978 among the main supplier countries — including Switzerland.

Finally the US has so far been unable to get very far in developing an aid package acceptable to the Pakistani government which would both guarantee Pakistan adequate protection against any Soviet threat but also persuade it to give up its attempts — still denied by Pakistani officials — to produce a nuclear bomb.

Faced with these interlocking problems, Mr Gerard Smith, Assistant Secretary of State for non-proliferation issues, has been actively lobbying Congress for a change in the legislation that would remove the NRC from, as one state department official puts it, "second guessing the President on foreign policy issues". □

The Netherlands

IN an energy memorandum designed to foresee the country's needs until the beginning of the next century, the Dutch government has recommended a drastic reversal of its post-war policy to run down coal production. From a pre-eminent position in 1955, when coal contributed 96% of power plant fuel, the mining industry declined as natural gas fields came on stream in the sixties and early seventies. By 1975, coal contributed only 0.7% of the country's power plant fuel stock, and even after the 1973 oil crisis (which led to the re-opening of mines in the south of Holland), it still contributes no more than 6%.

Now the government intends to boost coal production to a level where it supplies 40% of power plant fuel (12 to 14 million tonnes of coal a year) by the year 2000. Gas and oil, which currently account for 90% of power plant fuel, will be restricted to a 20% contribution, and a decision on where the remaining 40% can be found will be delayed at least until 1983.

At present there is an over-capacity in Dutch electricity generation, but this will disappear gradually as older plants are taken out of operation. Of the country's total of 15,000 MW installed capacity, 520 MW is nuclear, and although the government made a decision in principle six years ago to install three more nuclear

Dutch to fall back on coal

plants, discussions on specific proposals are expected to occupy at least two or three years yet.

An increased role for coal in industry is also foreseen in the memorandum, which talks of fluidised bed combustion accounting for the use of five million tonnes of coal in the year 2000. This is expected to require an investment of at least 1½ billion guilders (£1 = 4.4 guilders).

Eventually, new technologies of *in situ* gasification and hydraulic mining will be needed to exploit the 100 billion tonnes of coal lying beneath the entire country at currently non-viable depths of 1,000 to 6,000 metres. In the meantime, an investment of three billion guilders will be required for the gasification of 7½ million tonnes of coal annually for industrial fuel and chemical production.

This coal gas will also be used in a mix with high-calorific North Sea or Algerian gas, or even burnt in power stations — an area in which Shell and the utilities have already taken an initiative.

In a separate report, a group of Dutch industries has concluded that the role of

coal should be even bigger than the government intends, and they call for an expenditure of 25 billion guilders (in contrast with the official estimate of 10 billion) to get the coal industry into gear by the turn of the century. They want reserves to be re-exploited at a quicker pace, and they urge demonstration projects in gasification and fluidised bed combustion within the next two years. To see this combustion revolution on its way, the industrialists suggest, Holland should have a national energy fund and a special under minister for energy (which at present remains in the portfolio of the Minister for Economic Affairs).

A corollary of both the government's and the industrialists' dreams for Dutch coal is that research and development will be required to ensure that the environment remains as clean as it is currently on a gas-dominated energy regime. Anticipating an increase in coal use from 1½ million tonnes now to 26 million tonnes a year by the end of the century, the government is setting up a national R&D programme to concentrate on atmospheric problems, pressurised fluidised-bed combustion and coal gasification, while applications will be explored for flue gas desulphurisation work already carried out elsewhere.

Altogether, the research effort will cost 750 million guilders over the next five years.

Caspar Schuurin

International exchange

Scientific forum was not a great success

LAST month's Hamburg Scientific Forum was probably the first and last of its kind. For while the two-week meeting was useful as a platform for western participants to air their views on human rights, and for the eastern bloc served as a reinforcement of the notion that detente is still a going concern, it was in scientific terms virtually useless.

Certainly the UK government was unimpressed by the event. Speaking in the House of Lords, Lord Trefgarne has said that while "a number of useful conclusions" were reached at the forum, he could not give any general assurance about support for future meetings.

Earlier, the UK delegation said in its closing statement that the "practices and processes of the Conference on Security and Cooperation in Europe" were hardly suitable for establishing a framework of scientific meetings. Soviet participants were so embarrassed by the human rights debate which dominated the forum that

they are likely to demand strict limits on the agenda of any future meeting. In the event they will probably be saved the trouble. Member governments meeting in Madrid later this year are likely to shelve proposals for another forum.

While it lasted, the forum brought together well-staffed national delegations of experts prepared to put their heads together in order to discuss "interrelated problems of common interest" in medicine, the human environment and urbanisation. What they produced was not always original thought, but it was interesting as an indicator of the divergent paths seen by different countries as solutions to broadly similar problems.

The energy group, for example, found that the participant countries interpreted "alternative energy" in widely different ways. To the Soviet Union it meant primarily nuclear energy, with the emphasis on fusion research. Sweden stressed energy from biosystems, while Professor P. Boger (West Germany), urged the biological production of hydrogen as a means of using solar energy.

The food production group was hardly revolutionary in its findings, and called simply for "sustained R&D efforts in all aspects of the food system". International cooperation, it decided, was particularly needed in the development of plants with higher photosynthetic capacity, more efficient capability to use available mineral

nutrients and better ability to withstand environmental stresses. In particular, international efforts are needed in identifying and preserving plant and animal germ plasm in the natural ecosystems. More comprehensive gene banks should be set up "to preserve genetic materials for the benefit of plant and animal production in the future".

The medical group, dealing with cancer cardiovascular and virus diseases stressed the need for data sharing and the avoidance of unnecessary duplication. Further international cooperation, they found, is needed in such fields as standardization of diagnostic materials, recombinant DNA (including safety regulations and the evaluation of benefits) and large-scale studies (e.g. drug trials) where insufficient patients would be available in a single country.

Large-scale surveys of this kind, with comparison of the patterns of change in different countries were also urged by the social science (urbanisation) group. They suggested a regular programme or scientific review conferences and seminars, with special emphasis on the interdisciplinary approach. Yet, not even this group, keen as it was for meetings, suggested that a further 'scientific forum' was the appropriate setting. Rather, it suggested, the proposed meetings should take place under the auspices of UNEP, the ECE or UNESCO. □

United Kingdom

US carcinogen regulations urged in UK

ANIMAL tests should be relied on to indicate whether a chemical will be carcinogenic in humans, says a trade union document on occupational cancers published last week.

"When it comes to the interpretation of test results" says the document "the UK guidelines fall far short of their US counterparts. It is stated in the US guidelines that a positive result in animal tests is to be accepted as evidence of human carcinogenicity. Not so in the UK guidelines, where it is stated that 'extrapolation is a difficult procedure'".

Produced by Britain's half-million strong Association of Scientific Technical and Managerial Staffs, the report was launched at a press conference by ASTMS General Secretary, Clive Jenkins. "Too many of our members are dying" he said. "Tens of thousands of new chemicals have been introduced to the workplace since the war. Companies have acted irresponsibly. We want exposure to carcinogens reduced to zero. We want a new approach in law."

Sheila McKechnie, ASTMS Health and Safety Officer, said that her union "was not attempting to ban all chemicals". Of 7,000 or so chemicals that have been tested so far, says the report, "only 600-800 have shown any evidence of carcinogenicity".

Epidemiological studies — or 'body counts' as the report calls them — come too late: "keeping track of the effects of new (and existing) chemicals by observation of the effects on workers — if relied on exclusively — turns the work place into a giant laboratory for the conduct of an experiment in carcinogenesis. No trade union can accept this."

The evidence is that animal carcinogens are human carcinogens, says the report. "Even if subsequent research unearths some exceptions to this rule, it must be taken as a conservative regulatory principle, that is a principle erring on the side of safety. To refuse to accept the evidence of animal bioassays means, in practice, to insist on counting bodies as the only valid method of identifying carcinogens. This is the position usually adopted by industry — especially an industry threatened with the banning of some profitable commodity."

ASTMS is demanding a comprehensive licensing scheme for toxic substances. Under the scheme:

- Only potentially useful substances should be submitted for test.
- The tests should be carried out in public or independent laboratories and financed by a general levy on the industries supplying the substances.
- Only substances which pass these tests should be licensed for commercial use, subject to safeguards dictated by the results of the toxicity tests.



Jenkins: would love health strike

● All companies handling licensed substances should be compelled to keep comprehensive records.

The process of awarding compensation should also be completely overhauled, said Jenkins. "Some firms have internal compensation. We are urging our members not to accept it. One firm — I don't want to name it — went to a man on his death bed and persuaded him to accept the firm's compensation. He would have done much better in the courts."

ASTMS recommends that 'occupational cancer' be recognised as a broad category of industrial disease, and that cancer victims should be automatically entitled to compensation if they can show they were exposed to a known carcinogen, or were involved in an occupation that clearly shows an elevated risk of cancer. The scheme could be funded by a specially flouted National Cancer Insurance scheme, drawing on both public funds and general levies on industry. "There is great sympathy in the Trades Union Council for our views" said Jenkins.

Would ASTMS take strike action over

this issue? "I'd love a strike over health" said Jenkins.

However, the US regulations urged by ASTMS are themselves under pressure, as reported in *Nature* (24 January, page 320). In the latest proposals of the US Occupational Safety and Health Administration, it is expected that the list of 'category 1' carcinogens (ones where scientific evidence of carcinogenicity is strong) will have only 150 chemicals, and only 10 of these a year will be selected for 'special attention'. US industry still feels the regulations to be too restrictive, while unions are attacking OSHA for lowering its sights.

At its press conference, ASTMS was attacked on the scientific basis of its report, which claims that 20-40% of cancers can be traced to occupational causes or synergisms. In particular two of its sources have been roundly attacked: Samuel Epstein's book, 'The Politics of Cancer', and a joint report of the US National Cancer Institute, National Institute of Environmental Health Sciences, and the National Institute for Occupational Safety and Health, titled 'Estimates of the fraction of cancer in the US related to occupational factors'.

However, said Sheila McKechnie, ASTMS did not want their recommendations to stand or fall on technicalities. If the proportion was 40% or 5% it was still too much.

So how will British industry respond? "We are trying to get unions involved in testing these chemicals, to make sure they are unbiased" said Sheila McKechnie. "Initially testing will have to be done in industry; but so far we have met no resistance from companies on the pre-testing of new chemicals. It's chemicals where companies are already deeply involved where there is a problem."

Robert Walgate

Coal produced electricity the cheapest, says US economist

AN American energy economics consultant who waged a successful environmental battle against the emissions of coal-fired power stations in the early 1970s argued last week that electricity produced from coal in the late 80s will be cheaper than nuclear electricity. If he were right, break-even would only occur in the UK if coal tripled in real price over the next eight years.

The consultant, Dr Charles Komanoff, presented his views at a joint session of the Parliamentary Liaison Group for Alternative Energy Strategies and the

environmental group Green Alliance. (Parligaes is a rapidly growing association of MPs and others designed to provide a source of information in the House of Commons alternative to that of the national energy bodies in the case of electricity, largely the United Kingdom Atomic Energy Authority and the Central Electricity Generating Board.) Later, Komanoff gave evidence before the House Select Committee on Energy.

In both fora, Komanoff based his argument on an analysis of capital cost escalation in the 162 power plants built in

the US between 1971 and 1978. Of these, 116 were coal plants, and 46 light water reactors (of which 33 were pressurised water reactors — PWRs).

Over this period the coal plants suffered a real cost increase of 68%. Almost all of this increase, said Komanoff, could be accounted for by new apparatus to control emissions, such as SO₂ scrubbers (half the increase), improved efficiency in electrostatic precipitators and the like.

The nuclear stations built over the same period became more expensive at almost twice the rate. They increased by 128% in real terms.

"If we are to project these trends" said Komanoff "we need an hypothesis for the increases". In the coal case, the reason was environmental controls. In the nuclear case, "it is impossible to attribute specific costs to specific controls, as regulations ripple through the whole plant". The provision of emergency core cooling, a containment vessel, protection against fire, the provision of monitoring instrumentation, protection against seismic events — all had their effect on costs, he said.

A further hypothesis, he suggested, was that regulations become increasingly stringent to hold down total environmental costs. If the number of coal stations were doubled, there would be demands to halve their emissions. The same applies to nuclear power, Komanoff believes "to keep total risk constant, the probability per reactor of an accident must fall".

In both the coal and nuclear case, he calculated, station costs increased by 50% for each doubling of the corresponding sector.

Moreover, the lessons of Three Mile Island have yet to affect power station costs. "The Kemeny findings are not the end of the story. The Nuclear Regulatory Commission, and the Advisory Committee on Reactor Safeguards are constructing proposals which will lead to sweeping, thorough, and comprehensive improvements in reactor design." These alone would continue the upward trend in nuclear costs "and if a Welshman sees certain safeguards applied to a reactor in Virginia, he will want them in Wales too".

In the UK, coal station costs would also rise, thought Komanoff, because "at the moment you export all your SO₂ to Scandinavia and Germany" so there would be pressure for controls, but this would not be so strong as the purely internal pressure in the US — which has now had its major effects.

The net result, he said, was that a 1150 MW nuclear plant built in the US in 1988 would produce electricity at 4.74 cents/kWh. A 300 MW coal station built at the same date would produce electricity at 3.83 cents/kWh, allowing for interest on capital costs. The capital costs would be: nuclear, \$1684/kW; coal, \$945/kW.

But, said David Widdicombe, QC, who was chairing the Parliagaes/Green Alliance meeting, the crucial question was

whether there were reasons to expect the British experience to be different.

There were, said John Jukes of the CEBG. First, progress towards the coal production targets of the National Coal Board were slow; and coal will be needed for other purposes (such as substitute natural gas), pushing its price up. "The US has plenty of cheap coal, so relativities are bound to be different". But, said Komanoff, even a tripling of coal costs would only equalise coal and nuclear electricity prices.

Further, said Jukes, the UK has an appalling record on large construction sites and "until we get that right no-one will know the costs."

"Also the regulations in the US have been piled on one after the other in a not very efficient way." Britain should avoid a detailed licensing procedure like that in the US, said Jukes, where every component has to be certified.

"We won't know the costs until we place the orders for the next advanced gas-cooled and pressurised water reactors" said Jukes, backed up in this by a colleague Mr F P Jenkin, head of the system planning branch of the CEBG. "The CEBG has no commitment to build plant which are too expensive" said Jenkin.

However, a broad call was made at the meeting for the CEBG to expand on a term in its own costings, prepared for the meeting by Mr Jenkin, where the whole cost advantage of nuclear stations comes in a single item: 'net system savings'. (See table). This term contains most of the important assumptions, said Komanoff; will the CEBG not spell them out?

"There is a strong feeling in this meeting that more information is needed" said Widdicombe. "I will certainly accept that" said Jukes.

On the other hand David Pearce, of the Department of Political Economy,

University of Aberdeen, and a prominent critic of the Windscale inquiry into the expansion of nuclear fuel reprocessing facilities in Britain, attacked Komanoff's own assumptions. "The average load factor for a coal plant in the US is 55%, not the 72% he assumes. And I found his results are very sensitive to coals costs. If coal rose to three times uranium costs, his coal/nuclear balance would be reversed."

At the Select Committee on Energy, Komanoff was also subjected to close criticism. "If nuclear reactors are uneconomic" asked Arthur Palmer "why do the utilities continue with them?" Because, said Komanoff, they feel they are fighting a holy war. "But does that eliminate the profit motive?" asked Palmer. There is no profit motive, said Komanoff, because utilities' profits are guaranteed by state law, which guarantees an electricity rate which will cover running costs and give a return on capital investment.

Komanoff recommended that if Britain were to have an expanded nuclear power programme, it should not chose the PWR. There are more PWRs in the world than any other reactor type; "and if you leave yourself open to design reviews of PWRs the world over, you will constantly face escalating costs. On the other hand if you go for a minority reactor . . ."

"If I wanted to destroy the CEBG's nuclear programme" said Komanoff "I would recommend a large Westinghouse PWR". The average load factor of the 13 such reactors over 800 MW in the US was 44% last year. "And over 50 reactor-years the average load factor has been only 52%."

Smaller coal plants, of 200-400 MW, said Komanoff, should be able to operate at 70-75% "if the electricity supply system were properly planned".

Robert Walgate

Table: net effective costs of new stations (March 1980 prices)

	Nuclear			Coal Fired		
	(a)	(b)	(c)	(a)	(b)	(c)
Capital cost of station including initial fuel (£/kW) in the case of nuclear	850	1000	1150	416	490	564
Net effective cost (£/kWpa)						
Capital charges	71	84	97	35	41	47
Decommissioning	2	2	2	0	0	0
Other operating costs	12	12	12	10	10	10
Net system savings	-121	-121	-121	-30	-30	-30
Total	-36	-23	-10	+15	+21	+27

Notes:

- For both nuclear and coal fired stations, (a)×(c) represent -15% and +15% respectively on the central estimate of capital cost.
- Capital charges include those on associated transmission works.
- Capital charges include the annuitisation of interest during construction.
- Decommissioning costs include plant scrap value allowance.
- Other operating costs cover salaries and associated costs, repair and maintenance, rent, rates, insurance, etc.
- Net system savings are system savings after deducting station fuel cost.

NEWS IN BRIEF

Aid recommended for nuclear test victims

NUCLEAR weapons testing probably resulted in "a small number of cases of death or disease" among those living downwind of the test sites in Nevada during the 1950s and early 1960s, for which the government should be prepared to take responsibility, according to press reports of an investigation carried out by a White House study group.

A local paper in Nevada, the *Desert News*, said that the study group's 57-page report, currently under "active consideration" according to White House officials, recommends that legislation should be drafted to compensate those who may have developed cancer as a result of the testing.

However the group is said to oppose legislation proposed by Senators Edward Kennedy and Orrin Hatch conceding liability for radiogenic cancers among residents of the fall-out areas during the period of the tests with courts directed to set the damages payable. The group argues that a more restrictive proposal should be worked out.

Over 950 current and former residents of states surrounding the testing region have already filed over \$2 billion in claims against the government for injuries which, they claim, are directly related to the testing.

Inflexibility of Delaney Clause criticised

DR DONALD Kennedy, until recently commissioner of the Food and Drug Administration and currently provost of the University of Stanford, last week criticised the Delaney amendment — which forbids the use of any chemicals in food shown to have caused cancer in laboratory animals — as being too inflexible and in need of revision.

Dr Kennedy said that the clause in the FDA's authorising legislation codifies the hypothesis that there is no threshold

concentration below which a chemical does not cause cancer. And although this hypothesis "probably holds most of the time", Dr Kennedy said that he was "as certain as I can be of any scientific prediction that some day, very soon, some compound will be demonstrated to have a threshold level for cancer causation."

The Delaney clause was a good version of how society should deal with such risks, he said, but was suffering because of its inflexibility, leaving no room even for a convincing scientific demonstration that there is a safe level for some cancer-causing substance.

"Any law that purports to deal with science ought to leave room for scientific progress," said Dr Kennedy. "For that reason I favour altering the Delaney clause and similar provisions to reflect presumption rather than certainty — a presumption of risk that could be rebutted by a scientific showing that a certain level is indeed safe, or that the use of a particular kind of experiment actually overstated the risks."

Cracks discovered in reactor turbine blades

CRACKS have been discovered in the turbine blades of ten nuclear reactors constructed by the Westinghouse Corporation. Company officials point out that, since the turbines operate totally outside the nuclear plant, they are not considered to present a major source of safety concern at present.

Critics, however, claim that if a turbine disintegrated as a result of the cracking, fragments of steel blades could seriously damage the reactor vessel or a safety system. The Union of Concerned Scientists has asked that plants where serious cracks have been found should be shut down immediately for inspection.

The US Nuclear Regulatory Commission has identified cracks in the turbines of ten reactors, and has drawn up a list of 19 reactors that should be inspected for cracking during the next scheduled shutdowns. The commission is currently studying whether an earlier shutdown is warranted for any of the reactors.

ESA countries approve Spacelab cost overruns

THE member states of the European Space Agency voted unanimously last week to reaffirm their commitment to complete the Spacelab programme in spite of cost overruns. Under the initial Spacelab agreement, member states retained the right to withdraw from the programme if cost overruns exceeded 20%. The Spacelab programme board last week approved a further funding up to a 40% over-run after which another vote can be

taken. Only Italy decided to reduce its contribution (from 18% to 1%) and the remaining ten states have assumed the increased cost. The 1980 Spacelab budget now amounts to \$304 million.

In the meantime UK space scientists have raised objections to the Science Research Council's rejection of Spacelab experiments in the last round of budget applications. "They are reluctant to recognise that space experiments are costly. If this keeps up the UK will lose its expertise in building space hardware," said one of them. An SRC official said that the SRC judged every experiment on a cost effective basis — "it's a question of value for money".

Violence, barricades and arrests at Plogoff

MILITANT demonstrators barricaded all roads into Plogoff recently — the Brittany town in cap Sizun where a complex of four nuclear reactors is to be sited — after Mme Amelie Kerloc'h, deputy mayor of Plogoff had asked the townspeople "to make the commune an island inaccessible to the police". The barricades followed a week of confrontation with a 600-strong police force in which police bombarded demonstrators with teargas grenades thrown from helicopters and the demonstrators countered with Molotov cocktails and stones against massed police formations. An explosive charge damaged the Loc'h bridge and 14 demonstrators, all young workers from the vicinity, were arrested. A delegation of mayors and elected officials from the entire cap Sizun region has demanded the withdrawal of the police as a first step to defuse the situation.

US publishes science magazine in China

THE first issue of a new Chinese language science and technology monthly, *Science and Technology Review*, was launched last month in Beijing. Published by the Education and Science Society in the US, the magazine is edited by US scientists and overseas Chinese scholars including C.N. Yang, Professor of Physics at Stony Brook and winner of the 1956 Nobel Prize in physics. 100,000 copies of the first issue are being sold in 29 municipalities, provinces and autonomous regions. The 104-page first issue includes articles on comparative economic systems — Japan, Yugoslavia and the USSR — and an interview with Sheldon Weinberg and Steven Glashow winners of this year's Nobel prize in physics. According to Xinhua, the Chinese news agency, the magazine, which is the first foreign publication to be given wide circulation in China, is to be given special attention by "the decision-makers and administrators of the state".



FEATURE



Mahler's revolutionary study

THE 1970s saw a debate on the role of science and technology in the economic and social development of the Third World. The failure of the development strategies propounded during the fifties and sixties had become clear. It has been repeatedly pointed out that the benefits of development programmes are not filtering through to the poor majority; western technology often helps to perpetuate rather than reduce economic and social injustice.

No international organisation has taken this to heart more than the World Health Organisation (WHO). This is ironical, because WHO is probably the best and most respected UN organisation in terms of technical competence, and this competence lies in western-style health technology.

But WHO has now set the goal of 'health for all by the year 2000', and to achieve this, it is no longer advocating big hospitals, sophisticated medical technologies, and specialist medical professions. It is now talking about

The UN General Assembly recently approved the World Health Organisation's goal of health for all by the year 2000. Here, the architect of WHO's new policy, Director General Halfdan Mahler explains his thinking to **Anil Agarwal**

concepts like primary health care which are based on such hitherto taboo ideas as community participation in health services, self-coping and self-care, appropriate technology for health, appropriate health cadres like 'barefoot doctors' and traditional medicine. The UN General Assembly endorsed WHO's new goal and its strategy of primary health care in November. All governments are now being asked to formulate by June strategies to achieve that goal.

The radical change in WHO's approach

to health care has been described as the 'Mahler revolution'. Director General Halfdan Mahler has been a major force in the recognition that past health strategy has failed to bring any organised form of health care to more than half of the world's population living in rural areas and urban slums.

But the attempt at change has produced resistance. There is very little real political commitment, either in the developed world or amongst the elite of the developing world, to do much for the poor. WHO now finds itself saddled with idealistic international resolutions that few countries are really keen to implement back home, whether it is in terms of coming forward with the funds required to bring health care for all, or in terms of implementing those strategies and re-ordering domestic health priorities.

Lack of a real constituency is not the only problem. Even to prepare the intellectual framework for the change has meant antagonising such powerful actors

in the existing health drama as the drug industry, the baby milk powder industry, the tobacco industry, and not least, the medical establishment. The latter exists within WHO itself. Convincing WHO's own secretariat of the new mission has not been an easy task; Mahler ruefully refers to his "electric chair".

Mahler was frank about how WHO is coping with the stresses and strains of this change.

"The constitution of WHO speaks of health as a human right; that physical, social and mental well-being should be at the highest possible level, so it was in the constitution of this organisation that it should have not only a narrow technical mission but also a social mission. This social mission was, however, neglected. In the 1950s, we had the post-war hangover, the white man's guilt complex, that something ought to be done about the poor world. Isolated technical assistance programmes — lollipops, as I call them — were developed. Everybody tried to do his missionary activity in the Third World, including me. WHO did some excellent technical work but in a number of rather narrow fields. Then came the reckoning in the 1960s that it is all very well to do a little bit of malaria control, some little work on some other communicable disease, but it doesn't get health down to the grass-roots. So in the 1960s we had agonising questions such as: can WHO assume its real social mission?"

Mahler's strategy to bring the social mission to the fore has been simple: "I felt that the most important thing would be to invite the member-states to take their responsibility, whether they came from the North or the South. The big words, the collective decisions in the political organs of WHO, could no longer remain dead letters. This meant that the member-states had to ask WHO to be their bad conscience. We challenged them with the idea of health for all by the year 2000. It was not shot down in flames. The view was expressed that this indeed ought to be our aspirational target. This then raised the question: what should be the tool in moving towards that goal? That led to the Alma Ata conference on primary health care (PHC) in 1978 and the charting of the primary health care approach."

"There is now", according to Mahler, "in the governing bodies of WHO a realisation of what this movement towards health for all could become. Member states, however, often tend to forget individually what they have decided collectively".

The health debate has focused on two types of contradiction, says Mahler: "First, the conceptual contradiction. Previously we never dared to say that the end-product, health, should be attainable by everybody. We only spoke of services. We said we will try to get as big a coverage of services as possible.

"We have only a marginal degree of

popular participation in health care. This is the fundamental contradiction: if health doesn't start with the individual, the home, the family, the working place, and the schools, then we will never get to the goal of health for all. Even if we take the example of the industrialised countries, self-care, self-responsibility, self-coping in the individual, family and community, represent 50-60% of all care. Unfortunately, health professionals are rarely willing to trust people to such an extent that they acquire power to make the decisions that have to do with their own health. I just don't believe that anything in the North, for instance, can truly be changed when it comes to overeating, overdrinking, abuse of drugs, overstress

‘If health doesn't start with the individual, the home, the family, the working place, and the schools, we will never get to the goal of health for all’

and alienation, if we do not give power to the people to understand their own predicament, and then make conscious decisions about where they want to go."

In every country there is this fundamental contradiction between people and the medical consumer markets that they are provided with. "Of course", says Mahler, "the consumer markets in the developed countries are better because there you find, for instance, drugs all the time. That is because that is where the profits are. But if you go to a developing country, you find drugs available only for 3-4 months a year, which does not exactly generate confidence in the health services. Now by first looking at people's needs we know that we can no longer talk about putting drugs at random into the market. We must have a national policy whereby certain drugs are available all the time. Then we know we can get penicillin for the kid whenever he has pneumonia."

The second major contradiction is that the health services like to deal with only the top 10% of the health pyramid. Medical education is such that it teaches the new professionals to love the highest technology and medical research and development is such that it likes to generate only the highest technology. Resource distribution in the medical sector is such that 80-90% of the resources go to meet 10-15% of the health problems."

In his speeches to the World Health Assembly, Mahler has repeatedly emphasised the need for a strong political commitment if these contradictions are to be resolved. They can be found everywhere in the world. "I have been in two developing countries in the last few months," explained Mahler. "In one of them, the head of state asked me for help in fighting the vector the mosquito. I replied:

'Mr President, indeed we have been trying to do that, but I do not think that we have vector control programmes that can prevent what is probably more important to you, that is, 100,000 or more kids dying from malaria every year. So by all means fight the vector, but let us not forget that it is somehow more important for people that 100,000 of their children will die this year from malaria.

"Now this is not a play on words. By focusing on the vector, we could easily bypass our political responsibility. (The first thing that ought to be done is to organise a system through community participation, if necessary, by which everyone who has or is likely to get malaria can easily get a tablet to prevent it or cure it).

"In the other country, the prime minister asked that WHO's entire budget for two years be used for buying a body scanner. For the same money that country could immunise its children against measles thus saving half a million children dying in the next ten years."

But what is actually happening in the health ministries of the Third World? "I am not speaking of any miracles happening, but I am convinced that there is a reappraisal going on in many developing countries. This is setting into motion within these countries strong confrontations." WHO's role, as Mahler sees it, "is to enable member-States who find it politically difficult to cope with these confrontations to make use of WHO's neutral platform to cope with them.

"Look, for instance, at drugs. It was very difficult to get a national drug policy going in the developing countries because of the resistance, whether it came from the medical specialists or the general physicians. WHO, for better or worse, has been trying to get the best talent around the world and they have been saying that a concept like essential drugs is perfectly valid. Essential drugs that can cope with 95% of the problems even in relatively sophisticated societies number around 200. But for the Third World villager and urban slum-dweller great miracles can be achieved with 50 well-chosen drugs. It was WHO's neutral role as an arbiter that led to the realisation that there is nothing shameful in speaking about essential drugs. Now the onus is back on the governments. Can they now get their experts together and enforce this concept?"

In the confrontation over the marketing of baby milk powder, Mahler believes "WHO has played a unique role by providing a neutral platform. What we achieved was not a miracle, but when you compare it with the situation ten years ago, it was remarkable that industry, non-government organisations, and member states from developing and developed countries, could arrive at this kind of consensus. It was certainly a very



Halfdan Mahler:

‘Medical education is such that it teaches the new professionals to love the highest technology and medical research’

unpleasant kind of confrontation. I was very concerned about WHO getting involved in it. But slowly we picked up the courage and hoped that it would not end in a disaster. And it didn't. Now member-states will have a code of conduct for marketing of infant formulae. This will be a model which they can adapt to their conditions.”

WHO has also had confrontations with the scientific community, which Mahler says “finds it very unpleasant when you speak about social relevance and social equity. But take these one million kids dying in Africa from tropical malaria. Somehow, it must be possible to mobilise enough social commitment among those scientists involved in health research to say that this cannot be tolerated when we have such fantastic scientific tools today. Well, scientists initially said we are prepared to do our little bit as long as it is marginal. Now through our diarrhoeal diseases, tropical diseases and human reproduction research programmes, we are dragging them. We have opened our doors to the scientists. I think the response has been very good and will be increasing in the future.”

But is providing a neutral platform and creating a consensus enough in these confrontations? Is WHO happy with the speed with which the essential drugs programme is being implemented, for instance? “No, certainly not,” says Mahler. “But then we have to ask ourselves whether WHO isn't becoming a little megalomaniac in this field of drugs. WHO now finds itself fighting the world at large. We are now moving straight into technology, production, patents, trademarks, the elements of a new international economic order in the widest possible sense. Is this WHO's role? I think we have set the scene, and other organisations like the UN Conference on Trade and Development, the UN Industrial Development Organisation, World Bank, etc, should now play the key role. If you

look at the drugs scenario, despite its imperfections, there is already a lot of courage, in espousing the essential drugs idea, courage by a number of developing countries now developing national drug policies, and courage by member-states in the Pacific region, for instance, who have got together under WHO auspices to decide on collective purchasing so that they can negotiate with drug suppliers much more strongly than in the past.

“But still I am not happy with the speed of the essential drugs programme but that is because I am not happy with myself. I don't seem to have the necessary social or economic ability to accelerate the process. And, therefore, I want to involve UNIDO, UNCTAD, etc into whatever I can do, and the drug industry itself, by asking it what can be done. I can give you an example. It was rather startling. We had a conference and the industry spokesman said that the production of vaccines and sera is so competitive that they were losing interest in it. What conclusion can you draw from that? When you want health for all and want to prevent six million children from dying from tuberculosis, whooping cough, diphtheria, measles and polio every year, then you need to vaccinate 100-120 million children every year. In order to get that you need a vaccine price that is low. But when the price is low, you can't get the products. So my conclusion is that we can no longer treat these vital, essential components of people's health as normal commodities in the market place. They may have to be taken out of the market place and other ways found to produce these essential drugs. But all of this obviously generates tremendous contradictions.”

But what about the contradictions inside the house? To what extent has the Mahler revolution filtered down within WHO itself? Mahler has several times told his staff that WHO should not be an academy of health sciences but a centre for managing health.

Mahler agrees that there is an internal contradiction. “It is difficult to take the plunge from doing things that you do well, to continue to do them well but with a very much sharper relationship with this overall target of health for all. The secretariat is asking, are we not throwing out the baby with the bath water? We had all these good technical things. Is the gap not too big between all these expectations of a social mission and our limited but well-done technical mission? This challenging target of health for all set by the member-states collectively is probably the only way of bringing the high priests on their toes — the high priests inside the secretariat and in the individual member-states.”

Could not WHO's technical reputation suffer in the process? Could it not find that it has compromised its technical reputation as well as failed to achieve its social goal? Mahler believes that the danger is there, but it does not inhibit him.

“Take the case of smallpox eradication

which was first decided on in 1957. The internal technicians in WHO — and don't forget I am one of them — claimed for ten years on technical grounds that the job could not be done. However, in 1968 we turned the argument around and said smallpox shall be eradicated and the political commitment shall be taken for granted. We worked backwards to see how our techniques and our management would allow this political commitment to manifest itself, and how our technologies could do the job. And we succeeded.”

“Most people analysing the contemporary world will say, forget about health for all. They will tell you that you will soon have a nuclear war in several of the developing countries. If you look at the world without a feeling for this imperative, historical necessity to change, then obviously you will generate a climate of gloom, and then be discouraged by it. Inside WHO, we could have gone on with basic health services, some tuberculosis, some malaria work. But now we have forced ourselves politically into interfacing with the collective aspirations of our member-states.

“I do not think that there is one person who can say that this change requires less of him technically. I think it requires infinitely more scientific imagination, in order to lift our technological thinking out of our cocoons in such a way that it relates more meaningfully to this broader social perspective. But I do not believe that in the process any of the conventional technical activities will be lost. In fact, the large majority will have become infinitely better within the next 5-10 years and more relevant to our social targets. In recent years, we have been generating the various research programmes, the expanded programme for immunisation, and planning for clean drinking water and sanitation, with the target of ‘clean water and sanitation for all by 1990’. All these programmes have highly explicit objectives. These objectives are key components of primary health care. The programmes are helping people somehow to identify with the components of the overall strategy. So now you have within WHO a progressive improvement, from a kind of technocratic alienation to begin with, to an identification with the major programmes that will be filling out the various major gaps in primary health care.”

WHO must become more responsive to the realities of the world, he says. “I have been asking member-states: do you really think that a technocracy like WHO should continue to exist? Either you believe that you can make WHO big enough to assume this brokerage role at the global, regional and country levels, or you don't. If you do, then we have to make a lot of agonising changes, and there will be no peace. But if and when the member-states realise that WHO cannot assume that larger mission, they should have the courage to say that we

couldn't make the organisation swing around to what we all believe should be done, so let's get rid of it. Let us not keep the system for some spurious political motive."

But Mahler is optimistic on several counts: "I think that we have to a large extent successfully called the North-South bluff in health. And having called it, it is beginning to hurt. I know many politicians in the Third World are very uncomfortable with the present situation in which the elite is eating up 80-90% of the resources. There is a genuine debate on health in the industrialised countries too, though to a lesser extent."

Given the "hysterical conservatism" that is rampant in the developed countries, Mahler is still not sure to what extent they are prepared to help the developing countries achieve the goal. But he argues the South "now has a much better chance of moving towards self-reliance in health. Now it can challenge the North by saying that to achieve our collective goal of health for all which we all have collectively decided, we will do 95-97% of the job ourselves. Will you not let us have that 2-3% of support that is essential for us to make the best of our 95-97%?"

And why should the developed world help? Because in the process it would only help itself. "Take the case of smallpox eradication. Ironically, it is the rich world that is making, according to a moderate estimate, a profit of about a billion dollars a year — savings in vaccine production, in treatment of complications, in surveillance mechanisms, in the tremendous waste of time involved in having to make vaccination compulsory. Now I should have thought it moderately reasonable on my part to say that half of the profits should go back to the developing countries. They did it really for the sake of the developed countries. Take Africa. Their form of smallpox was not very serious. They could live with that. If you have a million children dying from malaria, it is not the most important thing to eradicate smallpox. But because of its commitment to global solidarity, Africa had to eradicate smallpox. It just shows how inter-dependent the nations of the world are in health."

When developing countries undertake research into their own problems, even then the West benefits. "Take my own little experience in tuberculosis," says Mahler. "In the 1950s, when I went to work in India, we were exporting the classical European notion of TB sanatoria. India then had three million cases of TB and little money to diagnose, treat or immunise. Well, the government of India picked up the courage to do research itself, and indeed the consequence was that the chemotherapy research that came out of the Madras TB research centre became internationally the most important. The centre succeeded in standardising the procedure of diagnosis, and in developing the concept of



Medicine in two worlds: primary care in Africa, high technology in the North



intermittent home-based treatment. All of this research was clearly geared to India's absolutely incredible problem, and these were studies that would never have been done anywhere else in the world.

"Yet, ironically, the great beneficiaries of all this research were the rich countries. Hundreds of millions of dollars have been gained in the developed countries by making use of the Indian research. A European country I recently visited has been able to avoid constructing 3,000 beds for TB treatment because they now have the WHO expert committee's recommendations based on research done in India that home-based treatment is adequate. This was the effect in just one developed country."

But probably what the developed countries will gain most is knowledge about how to handle their own health problems by emulating the approaches being developed in the Third World — a kind of reverse transfer of technology.

"Global standards of health and well-being are declining: life expectancy, after reaching a peak, is now again decreasing; cancer rates are rising; cardiovascular diseases are rampant; drugs, alcohol, cigarettes and traffic accidents nowadays

kill more people than did all the epidemics together in earlier centuries; the aged are overwhelmed with diagnostic tools and abstruse technology, but their psychosocial and mental well-being is left largely unattended and uncared for.

"This uneasy feeling about today's medicine is widespread. As a consequence of the present high technological pitch of diagnostics and therapeutics, the very attempt to diagnose and treat one disease may produce another. As many as 20% of hospital admissions are reported to fall in this category in certain parts of the affluent world. Our world has become too accustomed to believe that with a little bit of resources and a little bit of knowledge any problem can be solved.

"I think that an appreciation of the South's real health problems will have a better chance to emerge once the North gets a feeling for the incredible irrationality that exists in its own health system. This will be an additional factor leading to the true global solidarity necessary to achieve health for all the world's peoples. If you look at our concepts about the transfer of science and technology, then I have no hesitation in saying that we really are out among the *avant-garde*."

CORRESPONDENCE

Two good reasons to reject the PWR

SIR, — Sir Alan Cottrell (*Nature*, 28 February, page 804) has put his finger on the principal weaknesses of the PWR — the two-phase coolant and the pressure vessel failure problem — either of which is a cause for doubting the wisdom of adopting this reactor for the UK. Taken together they are sufficient reason for abandoning the idea completely. One might wonder why our electricity authorities are so keen to get into the PWR business at all.

Power station construction engineers are perhaps the main pressure group. They have not performed well with the AGR for many reasons, including starting construction long before the design was ready, and trying to develop and prove major components as they went along. The resulting delays and cost increases are well known, and technical difficulties have been introduced that have caused poor operating records in the early life of finished stations. Now, with remarkable lack of introspection, the same engineers are identifying the culprit as the AGR itself; amongst other things, they say, it is too complicated mechanically and needs too much work on site. They regard PWR as the solution to their problems (or should we say shortcomings), for they have convinced themselves that it is much easier to build than the AGR. This is the dangerous sort of complacency that led to the Dungeness B fiasco, which was very nearly repeated with SGHWR.

The AGR is based on sound principles, uses good materials of construction, and has excellent safety and operating characteristics. This is a recipe for success but it does need first class detailed design and project engineering. So does any other type of reactor. It is no good running away from the gas-cooled reactors because it is thought, wrongly, that the PWR is an easier option. This simply wastes our limited technical resources to promote a reactor with some frankly undesirable properties. The better course is to recognise our failings, capitalise on experience and concentrate our best professional efforts on making AGR the successful UK thermal reactor that it can now be.

Yours faithfully,

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Dr. C. P. Haigh was founder and for 14 years Director of the Berkeley Nuclear Laboratories of the CEBG. Subsequently he was responsible for overseeing the design of CEBG generating plant, including the nuclear reactors

Concentrating ethanol

SIR, — The amount of energy used in distilling off ethanol has not escaped attention to quite the extent that Taylor (*Nature*, 17 January, page 714) suggests, (see E. V. Anderson, *Chem. Engng News*, 31 July, 1978, page 8). In a simple still, nearly as much energy is discharged in the cooling water as is available from the ethanol. This waste of energy can be greatly diminished, at a price, by using multiple effect or vapour compression distillation units. Vapour compression is particularly attractive because ethanol could then be continuously removed from the fermentation mixture at low pressure so that its inhibiting action on yeast fermentation would be minimised.

There are various alternatives to distillation. The most interesting is the use of a semi-permeable membrane. Thomas Graham, in the paper (*Phil. Trans. Roy. Soc.* 151, 183, 1861) in which he introduced the word 'colloid',

mentions "the well-known bladder experiment of Sömmerring". He gives no reference, but explains that the permeability of bladder is selective and water evaporates from the outside so that more concentrated ethanol remains within. This may well have been a traditional technique in Europe.

Geoffrey Gorer (*Himalayan Village*, M. Joseph, 1938) says that beer is hung up in pieces of gut in Bhutan so as to make a more potent drink. Perhaps we still have something to learn from primitive technology.

Yours faithfully,

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Soviet biotechnology

SIR, — Recently there has been a number of comments in *Nature* about biotechnology. In the issue of 10 January, (page 123) there was an attempt to assess the world situation in which, for instance, Japan was quoted as a world leader in this field; we would not dispute this claim. However, that "most socialist countries . . . are somewhat secretive as to detail" is a slightly misleading conclusion.

Whilst we were preparing the Society for General Microbiology Symposium on Microbial Technology in 1979 it came to our attention that the Soviet Union and other communist bloc countries also were becoming increasingly active in biotechnology. In 1978, for example, President Brezhnev at the 25th Party Congress cited the microbiological industry as a key growth area in the Soviet economy, the aim during the current five-year plan being to develop microbiological industry four times faster than any other sector of industrial activities.

The degree of commitment to biotechnology in the USSR can be judged by reference to just one programme, namely single cell protein (SCP) production. In 1977 Academician N.M. Zhavoronkov announced that the production of fodder yeast from various internally available raw materials was being planned to make the USSR self-sufficient in protein feedstuffs for animals by 1990. The 1980 forecast for SCP production in the Soviet Union is of the order four million tons per annum, a figure that makes interesting comparison with the UK's largest programme, the ICI "Pruteen" project, which should come on stream this year and is expected to produce 50,000 tons per annum.

Yours faithfully,

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Sakharov's plight

SIR, — The recent action of the Soviet authorities against our distinguished colleague academician Andrei Sakharov provides another testimony about the increasing violations of human rights and civil freedoms in the USSR.

One can understand (though not agree with) the decree of the Presidium of the Supreme Soviet of the USSR depriving Professor Sakharov of his numerous decorations. For scientists, however, it is difficult to understand the meaning of the decision of the Council of Ministers of the USSR, by which all the state prizes Andrei Sakharov was awarded in the last 25 years, have been taken away from him. Is he supposed to pay back the substantial amount of money which accompanied each of these prizes? Does the Soviet government really think that its decree will make Sakharov's discoveries (like the physical

principles for fusion reactors) non-existent or even invalid? Or that Andrei Sakharov will, by the decree of Soviet government, cease to be the author of them? For the first time Soviet reality has surpassed Orwell's visions.

Internal exile to Gorky deprives Sakharov of one of the main rights and privileges of the members of Soviet Academy of Sciences to have the conditions for carrying on his scientific research. It is not clear yet, whether the Soviet Academy is going to propose the expulsion of Sakharov from its membership, but it should be made quite clear in advance that such a step would have most harmful consequences upon international collaboration and contacts in science.

Yours faithfully,

FRANTISEK JANOUCH

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Disposal of carcinogens

SIR, — The International Agency for Research on Cancer (IARC), with the support of the Office of Research Safety of the National Cancer Institute, has recently undertaken a programme of research on the disposal of laboratory wastes containing carcinogens, which will give specific instructions for destruction and disposal of the various carcinogenic wastes from laboratories.

We give here a brief résumé of a meeting of the working group responsible for establishing guidelines and priorities for the programme. We welcome comments, information related to similar work being carried out in other laboratories, and suggestions for active collaboration in the programme. As resources available for work of this nature tend to have a lower priority than original work on carcinogenicity, it is particularly essential that available knowledge is pooled.

In discussing a general strategy for control of carcinogenic waste, the working group:

- emphasised that experiments involving carcinogens should be designed in such a way as to minimize the quantities of hazardous material used;
- recognised that the scale of operations varied with different types of experiments;
- stressed that a waste treatment should yield for disposal products having minimum adverse biological or environmental effects;
- recommended that control techniques should, as far as possible, be carried out at the experimental site to minimize transportation, which may involve a public risk; and
- insisted that an experimental plan should contain the action to be taken in the event of a substance escaping from control in the course of an experiment.

A waste control strategy must take into account both the types of material for which appropriate treatment would be required, as well as their comparative importance in terms of risk of exposure during manipulation.

In discussing possible approaches to waste destruction, it was recognised that properly designed incineration techniques require careful attention to a number of variables, such as temperature of operations, air supply and feed rate. An alternative approach is to develop suitable chemical reactions which yield non-hazardous products.

From a list of carcinogens to be considered in a destruction programme, aflatoxins, nitrosamines and polycyclic aromatic hydrocarbons were selected as the immediate priorities for investigation. Alkylating agents, halogenated compounds, aromatic amines and hydrazines were also considered as high priorities.

Yours faithfully,

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NEWS AND VIEWS

View of Venus from Moscow

from D.M. Hunten

In this issue of *Nature* appear two papers (pages 243 and 244) reporting results from the Venera 11 and 12 entry probes, which completed their missions in December 1978. Earlier that month, four Pioneer Venus (PV) probes had also entered the atmosphere, the bus which carried them made measurements of the upper atmosphere, and the Orbiter commenced a long series of measurements expected to continue at least through 1980.

The Soviet and American probes made many measurements in common, as well as several unique to each. The current reports are in the latter category, and are of particular interest in complementing the Pioneer data as published in the 23 February and 6 July (1979) issues of *Science*.

Water vapour

Water vapour, an extremely important molecule, is difficult to measure reliably in small quantities. The PV mass spectrometer has not reported any measurements of H_2O except those stemming from the cloud drop that lodged on its inlet tube. The vapours from its decomposition and evaporation left little doubt that the drop was concentrated H_2SO_4 solution, but made it impossible to determine the corresponding abundances in the free atmosphere. The gas chromatograph reported rather large (in the context of Venus) amounts of water vapour, 0.5% at 44 km, a few kilometres below the cloud base. Previous analysis of Venus' radio emission observed from Earth, had set an upper bound of 0.1%, which pertains to the

bottom 10-20 km. The paper by Moroz *et al.* (this issue, page 243) reports far smaller amounts however, using optical spectrographic techniques.

The principal investigator, V.I. Moroz, is a well-known planetary spectroscopist, and the experiment is elegant both in its power and its simplicity. It consists of a filter-wheel spectrophotometer covering the wavelength range 430-1,170 nm with the very modest resolution of 30 nm. Included in its coverage are several bands of H_2O , which can be measured with assurance that most, if not all, of the absorption takes place in the free atmosphere. A serious complication arises from the presence of an optical 'cavity' between the clouds and the ground; some of the light seen looking upward has made one or more round trips over this whole path. With many measurements during descent, the effect can be allowed for, and the height profile of H_2O obtained. The mixing ratio is reported to be 20 p.p.m. (parts per million) near the surface, rising to 200 p.p.m. in the clouds. An earlier experiment on the same principle, aboard Venera 9 and 10, gave similar results but with considerably less assurance. It is also reported that a gas chromatograph on Venera 12 gave an upper limit of 100 p.p.m. below 42 km.

I, as a spectroscopist, am strongly inclined to believe the low abundances. Needless to say, the PV experimenters, led by Vance Oyama, are concerned about the discrepancy and have worked hard to resolve it. They can find no problem with their experiment, and point out that the amount of H_2O they observed would require a large and improbable source of contamination. An ordinary cloud drop, for example, would not be enough. So far there is no reasonable explanation, but

most workers tend to favour the optical results.

Other data from the spectrophotometer bear on the structure and properties of the clouds, and on the possible presence of a number of trace gases. Considerable absorption in the blue is apparent and could be explained by the presence of S_2 , Cl_2 , Br_2 , or NO_2 at a hundredth of a p.p.m. or less (depending on the gas). Much larger quantities of sulphur, previously advocated, are clearly ruled out.

Lightning on Venus

Before the recent encounters with Venus and Jupiter, we had no evidence about the presence of lightning anywhere but on Earth. Voyager photographed what appear to be lightning flashes on the night side of Jupiter (*Nature* 280; 794; 1979), supplementing the earlier but less direct evidence from Venus. The new paper by Ksanfomali (this issue, page 244) reports the results from the sferics detectors on Venera 11 and 12. (Sferics, a contraction of 'atmospherics', are the electromagnetic impulses from lightning discharges, commonly heard on an AM radio receiver.) Abundant signals were recorded from both probes, and a periodic modulation on Venera 11 suggests that the antenna pattern was being rotated past a distant source. On the hypothesis that the individual strikes dissipate the same energy as on Earth, the 'storm' was located at a distance of some 1,500 km. This modulation is perhaps the strongest evidence that the signals were not merely due to electrical discharge from the probes themselves.

Additional support comes from an experiment on the PV Orbiter, whose purpose was to study the properties of the plasma at high altitudes. Normally the ionosphere totally reflects all low-

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frequency radio waves, and sferics are not observed by satellites. But there are special circumstances where energy can propagate through in the so-called 'whistler mode'. These conditions, as measured by other instruments on the Orbiter, are thought to occur fairly frequently on the night side of Venus. When they do, the plasma-wave detector sees impulsive signals of a kind not normally encountered at orbital altitude. The instrument would need much better time resolution to obtain the length or shape of the pulses, but they are consistent with an origin from lightning and no other explanation has been suggested. Taken all together, the evidence for lightning on Venus is fairly convincing.

Ksanfomaliti also suggests that lightning could generate a mean nightside brightness 10^{-4} that of the day side. This source is put forward as an explanation of the 'ashen light', a phenomenon reported by many visual observers who see the whole disk faintly illuminated when the planet is in a crescent phase. It remains to be seen whether such suggestions and reports can be reconciled with the brightnesses observed from vehicles close to Venus.

The indications are that lightning is likely to occur in any substantial planetary atmosphere. Theories of electrification are faced with the need to explain its presence under a wide variety of circumstances and atmospheric compositions. □

Hammond found that progesterone secretion from granulosa cells *in vitro* taken from small immature pig follicles could be inhibited by prolactin. Short-term oestradiol treatment also suppressed progesterone secretion. However prolonged treatment with oestrogens (for 48 h) resulted in increased progesterone secretion and reversed the action of prolactin from inhibition to synergistic stimulation of progesterone. The authors conclude therefore that "oestrogens may regulate the divergent actions of prolactin in the mammalian ovary."

The basis of the oestrogen requirement for proliferation and maturation of granulosa cells is as yet unknown. Thus the change in response to prolactin seen in the present experiments may simply reflect the maturation of granulosa cells: prolactin will stimulate progesterone secretion from granulosa cells taken from large mature pig follicles. In this respect the effects of prolactin in the pig follicle may be different from those in the human where high levels of prolactin inhibit progesterone secretion from granulosa cells regardless of the stage of follicular development.

The importance of the direct inhibitory effects of high prolactin levels on steroidogenesis by granulosa cells in follicles before ovulation is emphasised by the fact that the rare cases of ovulation in hyperprolactinaemia, in particular that associated with lactation, is associated with an inadequate corpus luteum capable of secreting only limited amounts of progesterone (McNeilly, Howie & Houston, unpublished data). This suggests that prolactin-mediated inhibition of normal granulosa cell development before ovulation may explain the inadequacy of luteal function. This is supported by the observation that drug-induced hyperprolactinaemia in the luteal phase has only a slight effect on progesterone secretion from the developed corpus luteum whereas hyperprolactinaemia induced during the follicular phase results in the formation of an inadequate corpus luteum (Delvoye *et al.* *C. r. Seanc. hebdom. Acad. Sci. (Paris)* Ser D. **279**, 1463; 1974). McNatty (*Fertil. Steril.* **32**, 433; 1979) has provided more direct evidence in support of this. Raised levels of prolactin in plasma and ovarian follicular fluid were associated with a reduced number of granulosa cells and a marked reduction in intrafollicular steroidogenesis, not always apparent from the levels of circulating oestrogens.

Thus it seems that prolactin may have an important regulatory role in granulosa cell development within the follicle. Although present reports suggest that oestrogens may modulate the action of prolactin, the species difference in this action has yet to be resolved. The importance of understanding these mechanisms is apparent since hyperprolactinaemic blockade of steroidogenesis may in part explain the natural contraceptive effect of lactation in women and many other mammals. □

Paradoxical prolactin

from Alan S. McNeilly

LACTATION in many mammals is associated with suppression of ovulation as a result of the failure of ovarian follicles to develop to the appropriate stage. The widely varying suckling patterns in different societies (from very frequent short periods, for example in the !Kung peoples of the Kalahari, to infrequent longer periods of suckling) are all associated with suppression of normal gonadotropin secretion and of ovarian activity, and with raised basal levels of prolactin which increase further in response to the suckling stimulus (see McNeilly *Br. Med. Bull.* **35**, 151; 1979). As ovarian activity normally resumes once suckling ceases, either the suckling stimulus itself and/or the raised levels of prolactin may be responsible for the suppression of gonadotropin secretion and the consequent failure of normal follicular development.

The best evidence that the prolactin level itself may be responsible for ovulatory inhibition comes from cases of pathological hyperprolactinaemia which is the apparent cause of 20% of secondary amenorrhoeas. Unlike lactation, the raised prolactin levels in this condition may be due to an alteration in hypothalamic catecholamine turnover. The way in which prolactin itself may play a part has received scant attention; it may act on the hypothalamic-pituitary axis to suppress gonadotropin secretion, or may act directly on the ovary to suppress follicular development.

Evidence that prolactin is implicated in normal follicle development has been obtained from studies of progesterone

secretion from human granulosa cells *in vitro* (McNatty, Sawers & McNeilly *Nature* **250**, 653; 1974). Levels of prolactin equivalent to those seen during the normal menstrual cycle were essential to maintain LH-stimulated progesterone secretion. In contrast, high levels of prolactin equivalent to those present in lactation and pathological hyperprolactinaemia inhibited progesterone secretion, an inhibition which could not be overcome by the addition of gonadotropins (LH or FSH). A similar situation was apparent in the mouse where follicular progesterone secretion *in vitro* was also inhibited by high levels of prolactin (McNatty, Neal & Baker *J. Reprod. Fertil.* **37**, 155; 1976).

But how prolactin can have both an obligatory permissive role in progesterone secretion and an inhibitory effect remains unexplained. Studies by Veldhuis and Hammond (this issue of *Nature*, page 262) go some way to resolving this dilemma. Using short term *in vitro* studies of progesterone secretion from porcine granulosa cells they found that oestradiol 17β seems to modify the action of prolactin.

Previous studies suggest that development from a primordial to a Graafian (preovulatory) follicle is initially under the control of FSH and oestradiol. LH stimulates oestradiol and androgen secretion from the thecal layer of cells surrounding the developing follicle; FSH stimulates aromatisation of these androgens to oestrogens by the granulosa cells within the developing follicle. Both FSH and oestradiol are required for the proliferation of granulosa cells and antrum formation within the developing follicle and induce an increase in LH receptors on these granulosa cells. Prolactin is detectable within follicular fluid of all follicles and receptors have been detected on granulosa cells. Veldhuis and

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Between the galaxies

from M.G. Edmunds

WHO could ask for a better probe of the material between the galaxies than that provided by quasars? Situated at a range of distances, some very great, the quasars give off plenty of radiation to allow the absorption spectrum of the intervening gas to be observed on Earth. The use on the largest telescopes of very sensitive light detectors capable of working with high wavelength resolution has led over the past few years to rather good data on the absorption spectra of several quasars. Particularly successful has been a detector developed at University College, London. Data on six quasars obtained with this instrument have been used to deduce the existence and properties of clouds of intergalactic material (Sargent, Young, Boksenberg & Tytler *Astrophys. J. Suppl. Ser.*, **42**, 41; 1980). These gas clouds are thought to be pure remnants from the initial 'Big-Bang'.

A formidable array of statistical tests on the spectra identified two main sources of absorption. The authors concentrate their attention on the Lyman- α line of hydrogen which normally appears in the ultraviolet at 1,216 Å, but the expansion of the Universe redshifts this line into the optical region as observed from Earth provided the absorber is far enough away. The Lyman- α emission (and absorption) intrinsic to the quasar and its surroundings are of course at the highest redshift in the spectrum, but many other absorptions almost certainly attributable to hydrogen appear at redshifts smaller than that of the quasar's Lyman- α . In interpreting the origin of these lines, the observed family of lines arising from three-times ionised carbon (CIV) is important. Of the two distinct types of absorbing region which seem to be present, the first kind gives rise to these CIV lines and simply represents the outer regions of galaxies through which the light from the quasar passes on its way to us. There is good evidence that the outer parts of galaxies do contain sufficient gas at the right excitation state to provide the absorption, as the same CIV line is seen in the outer halo of our own Galaxy when looking at the ultraviolet spectra of sources nearby, but outside, our own system (for example the International Ultraviolet Explorer Satellite observations of sources in the Magellanic Clouds reported by Savage and de Boer *Astrophys. J. Lett.* **230**, L77; 1979).

But the other type of absorbing region which gives rise to Lyman- α but no CIV is new, and is suggested to represent true intergalactic clouds. The physical parameters of these clouds are inevitably uncertain but the proposed sizes are 2×10^7

solar masses and a typical linear dimension of 30 kpc. This extent is about the same as a medium-sized galaxy, but the density of the cloud is very much smaller, as that of a galactic mass will be more than 10^{11} solar masses. The gas in the clouds seems to contain no (or very few) heavy elements — that is, nothing more complex than helium, as no absorption lines of heavy elements are seen at the wavelengths expected on the basis of the redshifts of the hydrogen lines. This implies that the gas is a pure remnant from the Big-Bang initial synthesis which leads to production of hydrogen and helium, but very little else. This contrasts with the gas in galaxies (and their halos) which has been contaminated with heavy elements produced by nuclear reactions in stars and their supernovae explosions. From the relative distances of the clouds as implied by their redshifts, the clouds are not clustered together in space in the way that galaxies are. This is good evidence that they represent genuine intergalactic clouds, and are not associated in any way with existing galaxies.

The discovery of a very primitive kind of object in the Universe is an interesting one, but it must be pointed out that the clouds may have comparatively little influence over the evolution of the Universe as a whole. Their number per unit volume of space at present is only about five per cubic megaparsec, fewer than the observed galaxies and very much less significant in terms of mass. Thus these new objects certainly do not supply sufficient mass to close the Universe and make it eventually re-collapse on itself — a role often proposed for intergalactic material.

Sargent *et al.*'s analysis of the thermal state of the cloud gas implies that the clouds are too hot to be bound by their self-gravitation, and must be confined by an even hotter tenuous intergalactic gas. But it is significant that such a gas could still not be hot enough to provide enough X-ray emission to explain the diffuse background of X rays observed in space. (The initial results from the Einstein observatory imply that much of this background may anyway be due to the quasars themselves). The allowed cloud parameters are apparently rather tightly constrained — in these physical conditions a rather bigger or smaller mass than is suggested gives theoretical estimates that imply they would either collapse or evaporate. It is perhaps surprising that such fragile clouds have managed to survive to the present day — presumably they represent a small fraction of clouds of a whole range of sizes which formed early on in the evolution of the Universe. The other clouds either collapsed (and perhaps combined) to give galaxies and quasars, or else dispersed to become the diffuse intergalactic medium.

Can we see any of these primitive clouds near our own Galaxy? The International Ultraviolet Explorer Satellite provides an opportunity to look at the brightest quasar 3C 273, but as yet no conclusive evidence of suitable Lyman- α absorption has been reported, although the CIV absorbing gas of our own galactic halo is clearly visible. But the number density of the clouds would predict only three clouds in the line of sight to 3C 273 — so even a complete failure to detect anything would not be too surprising on statistical grounds.

Undoubtedly this study goes a long way to demonstrating that a large fraction of absorption lines in quasar spectra may be satisfactorily explained as not being intrinsic to the quasars themselves, but due to the outer parts of galaxies and to clouds in the intergalactic medium. Just what fraction of the remaining absorption lines can be attributed to gas driven out of the quasars remains to be seen. □

Electrically conducting polymers

from Paul Calvert

FOR some time people have been looking for polymers with metallic conductivity or with superconductivity. Sulphur nitride was found in 1975 to be a superconductor at low temperatures and sparked a great deal of research which now seems to have mainly fizzled out. At the moment polyacetylene seems to have potential as a metallic conductor and is the subject of much interest.

For a material to be conducting there must be a supply of charge carriers, electrons or 'holes', and an easy, high mobility path for them to follow through the material. Thus one class of polymeric conductors consists of conjugated chains, with alternating single and double bonds, such that the pi-bonding orbitals overlap to form a path along the chain. Examples of this are sulphur nitride $(-S=N-)_x$ and polyacetylene $(-CH=CH-)_x$. Alternatively, stacked planar aromatic molecules can provide an easy conduction path perpendicular to the molecular plane as in graphite. Hence the interest which developed at one time in the conductivity properties of DNA, where the stacking of the base pairs suggested that the molecule could be a 'one-dimensional' conductor.

Acetylene normally polymerises to a grey, insoluble, infusible powder with all the properties of brick dust, except price. However in 1974 T. Ito, H. Shirakawa and S. Ikeda at Tokyo Institute of Technology (*J. Polym. Sci. Chem.* **12**, 11; 1974) produced thin films by exposing acetylene gas to concentrated solutions of Ziegler

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catalysts. Films formed on the liquid surface consisting of *cis*-polyacetylene at low polymerisation temperatures and *trans*-polyacetylene at high temperatures. Electron microscopy (*Nature* **282**, 286; 1979) shows these films to be mats of fibres of about 20 nm diameter and low density, 0.4 g cm^{-3} compared with a theoretical value of 1.2 g cm^{-3} .

Shirakawa in conjunction with A.G. McDiarmid and coworkers at the University of Pennsylvania measured the electrical properties of these films (*Phys. Rev. Lett.* **39**, 1098; 1977; *Chem. Commun.* 578; 1977). The conductivity of the *trans* polymer is in the range 10^{-4} – $10^{-3} \text{ ohm}^{-1} \text{ cm}^{-1}$ which makes it a semiconductor and not particularly interesting. The *cis* polymer has a conductivity of about $10^{-9} \text{ ohm}^{-1} \text{ cm}^{-1}$. However on exposure to iodine vapour to dope the polymer with 68% iodine ($\text{CHI}_{0.22}$) \times the conductivity of the *trans* polymer increased to $40 \text{ ohm}^{-1} \text{ cm}^{-1}$. At 1% iodine the polymer took on a silvery metallic appearance and went through an insulator-to-metal transition. The *cis*-polyacetylene reached conductivities of 10^2 – $10^3 \text{ ohm}^{-1} \text{ cm}^{-1}$, 11 orders of magnitude above the pure polymer values.

A range of dopants can be used (*J. Am. Chem. Soc.* **100**, 1013; 1978) and, in the same way as in semiconductors, both p and n-type materials can be produced such that a simple diode is made. Recently experimenters have used arsenic pentafluoride rather than iodine as it is less easily lost by evaporation. If the films are stretched to three times their original length the conductivity as doped becomes anisotropic, 2,000–3,000 $\text{ohm}^{-1} \text{ cm}^{-1}$ parallel to the stretch direction and 100–200 $\text{ohm}^{-1} \text{ cm}^{-1}$ perpendicular. Because the film resembles a knitted structure it is not clear to what extent this reflects changes in inter-fibre contacts and to what extent it is due to internal structural changes. F.E. Karasz, J.C.W. Chien and coworkers at the University of Massachusetts in cooperation with McDiarmid have been looking at the effect of varying polymerisation conditions and have found increases in conductivity if films are prepared at low densities and then pressed (*J. Polym. Sci. Lett.* **17**, 779; 1979).

A wide variety of spectroscopic techniques has been applied to the doped films but there is still much to learn. R.H. Baughman and S.L. Hsu of Allied Chemical report finding I_3^- and I_5^- in iodine-doped films (*J. Polym. Sci. Lett.* **17**, 185; 1979). T.C. Clark and G.B. Street of IBM say that oxidation always occurs to produce ionised chains with the counter-ions intercalated between the chains. When the charge density is sufficiently high the charges delocalise along the chain and the material becomes conducting (Preprints, A.C.S. Honolulu Meeting, 1979; *Chem. Commun.* 1066; 1978).

A number of related systems have been studied. Poly(*p*-phenylene) $(\text{—}\text{C}_6\text{H}_4\text{—})_x$ has a

Role of hydrogen in amorphous silicon

from P.G. Le Comber

SINCE 1975, when it was first demonstrated that it was possible to control the electrical properties of amorphous silicon (a-Si) by substitutional doping (see Spear & LeComber *Phil. Mag.* **33**, 935; 1976), there has been a tremendous increase in the number of laboratories throughout the world working on both the fundamental properties of this material and its possible applications in solar cells, low-cost p-n junction diodes, thin film transistors, vidicon camera tubes and in electro-photography. With the recent announcement by the Sanyo Company of Japan that it is manufacturing calculators, watches and clocks powered by a-Si solar cells, we can expect this effort to be intensified still further.

The particular form of a-Si which has aroused all this interest is prepared as a thin film about $1 \mu\text{m}$ thick by the decomposition of silane (SiH_4) gas in an r.f. glow discharge. It is now known that this material contains upwards of a few percent of hydrogen and in fact many workers (but not all) believe that these relatively large concentrations are essential for the good electronic properties of these films. In view of the potential of a-Si in a wide variety of applications it is clearly important to understand the role of hydrogen in producing and possibly limiting its desirable properties.

In a recent issue of *Physical Review Letters* (**44**, 43; 1980), D.C. Allan and J.D. Joannopoulos, at the Massachusetts Institute of Technology, have calculated the electronic states and total energies of the various configurations in which H atoms could be bonded into the amorphous silicon network. Allan and

Joannopoulos claim that there are limitations in previous publications on this subject and conclude that strongly interacting nearest-neighbour silicon monohydrides have an important role, particularly in the interpretation of the photoemission spectra. They also find that the energy gap increases with increasing H content, in agreement with published optical data. Their results show that the valence band recedes rapidly with increasing H content but that the conduction band remains essentially unchanged. Possibly their most interesting conclusion, however, concerns the large ϵ_y peak in the density of localised gap states that is observed in field effect and other experiments. Since it is believed that it is these ϵ_y states that at present limit the performance of a-Si solar cells, by understanding the nature of these states one might be able to reduce their numbers. Allan and Joannopoulos suggest that local fluctuations in the hydrogen content can create localised states near the valence band edge and that these could be the origin of the ϵ_y states.

It would be ironic if hydrogen, thought by many to be essentially only a positive factor in producing the good electronic properties of a-Si, turns out to be responsible through these fluctuations for limiting the performance of a-Si solar cells! This suggestion cannot be the whole story, however, since a-Si produced by thermal evaporation contains essentially no hydrogen and has an even larger number of states around ϵ_y than the films produced from silane. Nevertheless many laboratories will be interested in seeing if this suggestion by Allan and Joannopoulos provides, at the least, some clue to how to decrease the number of these states and thereby improve the efficiency of a-Si solar cells.

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conductivity of $10^{-14} \text{ ohm}^{-1} \text{ cm}^{-1}$ which rises to $100 \text{ ohm}^{-1} \text{ cm}^{-1}$ with arsenic pentafluoride. This polymer is a very stable powder (*J. chem. Phys.* **71**, 1506; 1979). Poly(vinylphenylene) $(\text{—CH=CH—C}_6\text{H}_4\text{—})_x$ has a conductivity of $10^{-10} \text{ ohm}^{-1} \text{ cm}^{-1}$ rising to $3 \text{ ohm}^{-1} \text{ cm}^{-1}$ at 57% arsenic pentafluoride. This material has a degree of polymerisation of only eight, barely a polymer at all (*Polymer* **20**, 1441; 1979). Polydiacetylenes $(\text{—CR=C=C=CR—})_x$ are of interest in that the polymers can be prepared as large single crystals by solid state polymerisation of monomer crystals where the monomers are already aligned in the right way for chain formation. Despite the chains being continuous through the crystals and their metallic appearance they

are not conductive as apparently they have no charge carriers (*J. Polym. Sci. Phys.* **14**, 2037; 1976).

Simple models suggest that in long conjugated chains with all the bond lengths equal, electrons should be thermally excited into the conduction band at room temperature. This does not happen, apparently because all the bond lengths are not equal (due to the Jahn-Teller effect, for the cognoscenti) and because internal rotation about the bonds interrupts the conjugation. In addition to understanding charge mobility within the chains one needs to know how the charges transfer from one chain to another, which must partly be a function of chain length. Unfortunately this is all but unmeasurable since the

polymer is insoluble.

Polyacetylene is a tractable material because it can be produced as films; there is no prospect of further melt or solution processing. It is possible that acetylene might be induced to polymerise as a single filament in the same way as a silkworm produces silk, also infusible and insoluble. Alternatively films could be rolled up to form wires but this would hardly be cheap. More probable is that acetylene could be polymerised directly onto the surface of integrated circuits to provide controllable resistance elements. Perhaps the most intriguing aspect of this work is that it requires cooperation between theoretical chemists, physicists, polymer chemists and engineers. Communication may be difficult but the number of journals in which it can be published is immense. □

Modern refuse and ancient behaviour

from Mark Maltby

THE connection between a caribou hunting group in Alaska, Hottentot occupational debris, the contents of trash cans in Tucson, Arizona and animal bones recovered from prehistoric archaeological sites may seem tenuous but they all come under the umbrella of ethnoarchaeology, a discipline that has assumed increasing importance in archaeological studies over the past 15 years.

Ethnoarchaeology attempts to interpret the behaviour of a society by means of its material culture. By trying to understand the relationship between these in present-day societies it may be possible to reconstruct the behaviour patterns of prehistoric groups by studying their archaeological remains. There is nothing new about archaeologists turning to the ethnographic record for inspiration but most anthropological fieldwork on the other hand, has tended in the past not to record in detail the artefacts, refuse, house and settlement plans, which often provide the only source of evidence for archaeologists. Consequently many American archaeologists took it on themselves to collect data more relevant to their own studies. There is now a rapidly increasing literature on ethnoarchaeological case studies from all over the world, mostly on hunter-gatherers but also on nomadic pastoralists, sedentary farming groups and even industrial societies. Analyses of the production, form, variability, use and discard of artefacts such as stone tools and pottery have been made. Various aspects of subsistence activities, the internal

organisation of settlements, the correlation between social status and material culture and the regional patterning of settlement sites and other activity areas have been studied. Several recent studies have concentrated on food refuse, animal bones in particular, and these can be used as examples of the scope of ethnoarchaeology and its potential for helping to understand the past.

Most animal bones recovered from archaeological sites were discarded as refuse but surprisingly little is known about the processes that determined the observable patterns of bone fragments and other refuse in excavated deposits. The need for a greater awareness of such processes prompted several studies of modern rubbish disposal. Perhaps the most ambitious of these — and in many respects the most unusual — has been the Garbage Project of the University of Arizona (Rathje in *Explorations in Ethnoarchaeology* (ed. Gould) 46, University of Mexico Press, Albuquerque, 1978). In this project rubbish from several hundred households in Tucson, southern Arizona was systematically sorted. The garbage has been put into more than 150 categories representing food, drink, drugs and so on and further relevant information about each item has been noted. For example, the weight, type, cost and brand of discarded food or its containers are all recorded. The analysis of this data is incomplete but the potential of such a survey for elucidating the social patterns underlying refuse disposal in a contemporary urban community is of value to archaeologists as well as social scientists.

Other ethnoarchaeological work has examined in detail the processes of fragmentation and preservation of animal bone. One of the earliest examples was that of Brain (*Scientific Papers of the Namib Desert Research Station* 39, 13; 1969), who examined discarded goat bones from recently abandoned Hottentot villages and was able to show that certain bone fragments had survived better than others and that preservation was linked to the density of the bone and the age of the animal to which it belonged. For example, sturdy fragments such as the distal portion of the humerus survived better than the more fragile proximal portion of the same bone. He used these data to refute Dart's claim (*Transvaal Mus. Mem.* 10; 1957) that the animal bones associated with australopithecine remains at Makapansgat, South Africa, had been manufactured as tools. Brain showed that the uneven representation of these bones was more probably due to differential preservation of their skeletal parts and not to any 'hominid' behaviour. Recently a more elaborate examination of the Makapansgat data has supported Brain's findings (Binford & Bertram in *For Theory Building in Archaeology* (ed. Binford) 77, Academic Press, New York, 1977). The problems of differential preservation have often been

ignored by archaeozoologists and it is only through these and similar studies that advances have been made in our knowledge of the preservation of archaeological bone samples.

The most detailed ethnoarchaeological case study on animal bones published has been carried out on the Nunamiut Eskimo in north Alaska (Binford, *Nunamiut Ethnoarchaeology*, Academic Press, New York, 1978). The Nunamiut rely principally on hunting caribou and Dall sheep for their food. Caribou are particularly important, providing the Nunamiut not only with most of their meat but also skins, marrow and bone grease. Most of the caribou are hunted during their spring and autumn migrations through the area inhabited by the Nunamiut. After a successful kill, the complete carcass may be transported to the village or skinned and more or less dismembered at the kill site and cached in the snow to be collected and possibly butchered further at a later date. Sometimes parts of the carcass may be abandoned at the kill site and never brought to the village. Some meat is required for immediate consumption in the village but most is stored either by freezing in cellars dug into the ice or by drying on racks. Further butchery may take place then and later during cooking. Certain bones are also smashed up to manufacture bone grease; others are broken to obtain marrow and some are thrown to the dogs.

All this transforms the skeleton of each caribou into a large number of distinct bone fragments scattered over several sites. The variability in the treatment of different carcasses is also reflected in the types of bone fragments deposited on the kill sites, hunters' camps and resident settlements. By combining his observations on Nunamiut behaviour with calculations of the value of the different bones for meat, marrow and grease and their general utility, Binford was able to construct models that could predict the types of bone fragments deposited at the different sites and examine the variability caused by the complex interactions of logistic, storage, mobility, seasonal and social strategies of the Nunamiut.

How can this and other ethnoarchaeological studies be applied to archaeological problems? If nothing else, they have demonstrated the potential complexity of animal bone assemblages — a complexity that has not been appreciated by most archaeozoologists and many statements that have been made about the proportion of different species represented, the relative abundance of their different skeletal elements, the kill patterns of the animals exploited and other aspects of archaeological data must now be viewed with suspicion. Ethnoarchaeological studies are, however, of most value if their information can be used constructively and Binford, for example, has claimed that some of the hypotheses generated and tested on the Nunamiut

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bone assemblages can be applied to most archaeological samples. It is now necessary for archaeozoologists to adapt such hypotheses to their data. If such

adaptations are successful, the depth and range of information obtained from archaeological animal bones will have been greatly increased. □

Compartments, 'switches', 'programs' and vertebrate development

from Jonathan Cooke

It is now well established that in insect development small groups of founder cells are set aside in a hierarchically arranged series of geographical partitioning events such that each group is subsequently solely responsible for the construction of a defined piece of the epidermal body pattern. These pattern pieces, each the product of such a 'polyclonal compartment' set up in the embryo, need not correspond to morphological units as recognised by an anatomist. They are however identical and similarly positioned with respect to each other in each individual. This finding has formed an exciting link between the study of positional information (Wolpert *J. theoret. Biol.* **25**, 1; 1969) that is, the control of cells' activities according to their relative positions within the embryo during morphogenesis, and the study of possible mechanisms for progressive restriction of cells' potentialities during early development (Crick & Lawrence *Science* **189**, 340; 1975; Morata & Lawrence *Nature* **265**, 211; 1977).

There has naturally been speculation that what has been revealed in insects, with their intricate and distinctive body surface and their suitability for the somatic genetic marking of cell clones during development, is in fact general to many types of embryo. Methods now exist for marking clones over long developmental periods by injecting

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the founder cells with 'heritable' substances (Weisblat *et al. Science* **200**, 295; 1978; Jacobson & Hirose *Science* **202**, 637; 1978), and first attempts to probe for compartmentation in vertebrates have been directed at brain development. Here, a complex and exact anatomy is finally produced and there is little reason to suppose that populations of cell bodies intermingle on a large enough scale, at late stages, to obscure any earlier geographical organisation. The idea of a sequential, hierarchical series of events channelling the fates of cells early in development is a comprehensible and attractive one. What are the chances of being able to detect compartmentation in the vertebrate brain rudiment if this exists — or, more fundamentally, what meaning could the concept of polyclonal compartments have there?

Regulative embryos such as those of vertebrates are those in which the body pattern progressively becomes determined within a numerically expanding, sheet-like population of cells, and not those where lineal descent seems to define cells' fates from the outset as in the nematode worm or the leech. In one trivial sense all regulative embryos ultimately exhibit a compartmentation of the founder cells for each element of their bodies during undisturbed development. But vertebrate embryogenesis contrasts rather sharply with that of insects in the timing of the restriction process relative to the onset of overt

differentiation.

In the insect work, the dramatic outcome of clonal analysis (the exploration of development by marking all the epidermal cells coming from single founder cells picked out at particular times) has been the revelation that precise restrictions occur long before overt differences become apparent among the cellular descendants. The restrictions do not apply to which histological structures those descendants can differentiate into, but to which region of the body's pattern they can participate in constructing. Very many cell generations then intervene, during which the commitment is only expressed in subtle ways such as growth rates, or the lack of intermingling that actually preserves the integrity of the compartments. It is precisely the failure of the undifferentiated cells to invade alien pattern regions that lends the compartment concept its interest and meaning.

In vertebrate embryos however, especially in the primordial nervous system, there is a wealth of evidence that commitment to pattern construction on the part of small cell groups occurs relatively late in the cell lineage, when cell number is high, and predates hardly at all the onset of morphogenesis and the first stages of histological differentiation (for example, C.-O. Jacobson *Zool. Bidr. Uppsala*, **36**, 73, 1964; Chung & Cooke *Proc. Roy. Soc. B.* **201**, 335; 1978). Certain very early events, such as the separation of the embryo from its supporting structures in mammals, are exceptions to this. It is true that the decision by a population of cells within the amphibian ectoderm to form nervous system rather than epidermis occurs somewhat earlier than does regionalisation for brain structures within that committed population. But even the former decision is certainly delayed until the embryo contains more than 10,000 cells, and the ectodermal cells concerned seem on average to be in their 15th division cycle since fertilisation (Suzuki & Ikeda,



100 years ago

Who are the Irish? By James Bonwick, F.R.G.S. (London: David Bogue, 1880.) This little work is issued as the first of a series on "Our Nationalities," to be followed by three others on the Scotch, Welsh, and English. It does not appear from the prospectus whether the rest of the series is to be entrusted to Mr. Bonwick; but if they are it is to be hoped that he will qualify himself for the task by a preliminary study of at least the first principles of ethnology. The present volume, with all its good intentions and praiseworthy industry, must be regarded as a hopeless failure, owing entirely to the neglect of this necessary precaution. For many years ethnology, anthropology, and philology were subjects

which anyone seemed competent to deal with, who had got hold of a few lists of words in some obscure African or Polynesian dialects (the obscurer the better), or who had desecrated a sufficient number of ancient barrows, or posed to admiring circles under the shadow of some Druid's altar in Cornwall or Brittany. But those halcyon days of the amateur ethnologist are no more, though the writer, unfortunately, seems scarcely alive to the fact.

The following epigram on Dr. Siemens's recent paper has been sent us as by "a well-known scientific man". It is entitled Electric Chlorophyll:—

"Quis veterum vidit plantas sine sole virentes
Germinat en semen Siementis lumine claro."

It appears that the Berlin Municipal Corporation has granted to Dr. W. Siemens the concession of one electrical railway which will connect Wedding-Platz with Belle Alliance-Platz. The rails will be supported by

iron columns, which will not be an obstruction for the circulation of carriages and passengers in the streets. There will be no intermediate station between the two termini.

A deplorable accident has taken place at the Grenoble Lycée. The professor of chemistry was lecturing on salts of mercury, and had by his side a glass full of a mercurial solution. In a moment of distraction he emptied it, believing he was drinking a glass of *eau sucrée*. The unfortunate lecturer died almost immediately.

Mercury was seen at Paris on May 10 and 11 with the naked eye, owing to the transparency of the atmosphere and the great elongation of the planet. It had the brightness of a 1st class star, and was of a yellowish colour. The observation was made by M.M. Henry brothers, at the Paris Observatory.

Etna is again tranquil, its summit is once more covered with snow, and an ascent is contemplated, with a view to examine the alterations caused in the crater by the recent eruptions.

From *Nature*, **21**, 18 March 1880, 473-4; 1880.

Dev., Growth Differ. **21**, 175; 1979). The placement in cellular terms of the boundary between future brain and epidermis may be altered by grafting (for example Spemann & Mangold *Arch. Mikr. Anat. u. Entwmech.* **100**, 599; 1924) or by artificial imposition of physiological gradients up to this time, so that a recent claim that seven true compartments within brain structure are established within the 512-cell stage amphibian, besides being unsupported by the data produced (M. Jacobson *Trends in Neurosciences* **3**, 3; 1980), is incomprehensible and at odds with all other understanding.

There is no reason to believe that vertebrate groups other than amphibians are any more precipitate in marking out their brain plans. But most important, the means for a temporal analysis of the logic of cellular commitment, when it does take place, are denied us in these animals. This is because transition from pluripotency to quite detailed commitment within small pieces of neural tissue proceeds very rapidly in relation to the cell cycle. Cell number scarcely doubles in the neural plate and tube during the essential establishment of the brain's pattern. Marking clones will thus be of no avail in finding out whether in fact a telescoped version of the binary, sequential decision-tree that has so fascinated the *Drosophilologists*, also occurs among the cells of frog, chick and mammal embryos.

Closely associated in the insect literature with the idea of compartments is the concept of the 'selector gene'. This is based on the existence of a class of mutants called *homoeotic*, in each of which the cell populations that should belong to a particular set of the compartments instead show a tendency to construct, and thus duplicate, pattern regions proper to other compartments. Thus in one case, cells in the posterior of the anterior/posterior pairs of compartments that construct the wings (and also the obviously homologous 'halter' structures of the succeeding body segment), show a pronounced tendency to duplicate instead the structures proper to the anterior compartments, while in another case, antennal structures tend to be replaced by the leg pattern. Homoeotic mutations are now widely assumed to be in genetic units — the selector genes — whose wild-type function is literally turned on as a positive switch to define and maintain membership in one of the two compartments segregated by each restriction event at particular levels in a hierarchical sequence or 'program' (Garcia-Bellido *Am. Zool.* **17**, 613; 1977). Pairs of compartments are generated in parallel sequences from previously established ones, so that membership of each final compartment would be defined, simply and combinatorially, by the states of the set of on-off switches represented by the selector genes.

Again the idea, with its attendant image of the 'computability' of development, is

at present an attractive one. There is little doubt that the phenomena of polyclonal compartmentation and of homoeosis have revealed some sort of logical and combinatorial structure for restrictions on the potentialities of groups of cells during development. But I feel the selector gene model to be more an example of what Stent (*Trends in Neurosciences* **3**, 49; 1980) has termed the ideological component of the molecular genetic viewpoint, than anything logically required by the data. Shouldn't we do well at this stage to be flexible, rather than succumb to the current tendency for each aspect or event in an organism's life that attracts our interest promptly to become the express responsibility of a gene? Parallel manifestations of this incipient social disease in psychiatry and criminology need no comment, but they may also be seen in contemporary evolution theory (for example Dawkins *The Selfish Gene*, Oxford University Press, 1976; for a critique of the syndrome see Gould & Lewontin *Proc. Roy. Soc. B.* **205**, 581, 1979).

It was the great success of molecular biology to reveal that conservation and readout of the information that specifies proteins, and at least in prokaryotic cells, control of that readout, resembles in form the programs we design to retrieve and use information. Ideology only creeps into our view of development when it is supposed, without evidence, that the reliability and constancy of the phenomena seen there reflect an extension of the same sort of programming that controls the biosynthesis of the basic building blocks and signals. The complex and repeatable sequence of readout from the genetic library that accompanies development does not allow us to conclude that the process is actually being driven along by a digital program embodied in individual genes as molecular switches.

No experimental results yet require that any episode in the setting up of the body plan of any animal is literally the responsibility of a particular piece of DNA. The *bithorax* series of mutations in *Drosophila* (Lewis *Nature* **276**, 565; 1978) all occur in one short stretch of chromosome in a spatial order that could reflect the series of homoeotic transformations they cause between the compartments underlying the segmental plan of the insect. Here we have a hint that organisation of a stretch of genomic information may directly reflect the organisation of development, but specific selector genes need not be invoked as the targets for all or even most homoeotic mutations. Cells seem to be systems with a few stable end-states or pathways of development and a defined sequence of possible transformations between these, whether driven systematically or by chance perturbations (see Hadorn in *Major Problems in Developmental Biology* **85**, Academic Press, New York, 1967, for

other relevant phenomena in *Drosophila* cells).

Waddington (*SEB Symp.* **2**, 145; 1948) and Kauffman (*J. theoret. Biol.* **22**, 437; 1969), among others, have discussed how these observed properties might emerge at levels far removed from single genes as switches. The genome will surely contain many targets for mutations which modify various properties of all the developing cells (such as membrane transport, division rate or responses of control mechanisms to small molecules) in ways that are limiting only to their entry into certain of the available epigenetic states (that is, compartment designations). We might then expect these less available states or designations to be replaced on a systematic basis by alternative ones. Until direct molecular knowledge is at hand that justifies us in replacing views like those outlined above with the notion of a more direct programming of development written into the structure of the genes, our phenomenological knowledge about the regularities of development should be recognised as being just that. □

Continents old and new

from I.G. Gass and J.B. Wright

MANY geological texts and research papers identify two schools of thought when it comes to the subject of the origin and evolution of continental crust. One school, which could be labelled the 'conservationists' or 'reworkers', maintains that virtually all of the continental crust formed during the earliest part of the Earth's history and has been periodically reworked since. Reworking involves remelting and recycling of the original crust but not adding any new increments to it. The alternative 'accretionist' or 'incremental' hypothesis is that continental crust has grown continuously or episodically since the time of preservation of the oldest crustal rocks around 3,800 Ma ago.

A related question is the extent to which plate tectonic processes have operated during the Earth's history. At one extreme is the view that as soon as crust formed at the Earth's surface it was being moved about by plate tectonic processes; at the other extreme are those who maintain that plate tectonics did not begin to operate until late in the Proterozoic. While the meeting seemed to throw up a consensus favouring an incremental model for crustal evolution, opinion about the time of onset of plate tectonics seemed to be as divided as ever.

Several speakers provided evidence to support the thesis that (1) some 70–75% of continental crust was formed during the Archaean (> 2,500 Ma), the remaining

*A discussion meeting, 'The origin and evolution of the Earth's crust', was held in London by the Royal Society on 21–22 February, 1980 and organised by S. Moorbath and B. Windley.

25–30% during the Proterozoic and Phanerozoic; (2) crust-forming magmatic products originated in the mantle; (3) existing continental material had relatively little effect in modifying the composition of magmatic mantle products. Some took plate tectonic processes back to 3.8×10^9 years and correlated Archaean greenstones with Phanerozoic ophiolitic sequences and Archaean granitoids with products of present-day arc systems. Others pointed to geothermal and geochemical differences and argued either that if plate tectonics had existed in the Archaean, the processes were markedly different from those of the present; or that some other mechanism must have been involved.

Some interesting ideas were presented and several speakers dealt with particular aspects of accretionary processes. For instance, J.F. Dewey (Albany, New York), a staunch advocate of Precambrian plate tectonics, invoked the term 'para-oceans' and maintained that mantle processes leading to the formation of basins or para-oceans without reaching the true sea-floor spreading stage, probably make quantitatively important contributions to evolution of the continental crust. W.R. Dickinson (University of Tucson) suggested that sufficient variables exist in present-day plate tectonic processes to explain all the differences identified for older geological formations. Others, notably D.E. Karig (Cornell University), A.M. Ziegler (University of Chicago), W. Hamilton (US Geological Survey, Denver), F. Barker (USGS, Denver), R.S. Thorpe (Open University) and G.C. Brown (Open University), discussed the role of existing crust in modifying mantle products either by the incorporation of subducted sediments or by reaction between ascending magmas and the crust. Most argued that the modifications were quantitatively minor, though it was evident that a fuller understanding of island arc and back-arc processes (J. Tarney, University of Leicester) is essential. In this respect, B. Windley's (University of Leicester) report on an upended Upper Cretaceous arc in the Himalayas (traversed by the new Karakoram Highway), was particularly appropriate (see box). By way of a corrective to those advocating large scale relative movements of cratonic blocks in the Proterozoic, D.J. Dunlop (University of Toronto, Ontario) pointed out that the palaeomagnetic record remains inconclusive.

Geological, geophysical and geochemical modelling attempts to place restraints on the shape, form and composition of continental crust and hence on the processes that form it. E.R. Oxburgh (University of Cambridge) and P.R.A. Wells (Shell, The Hague) presented thermal models relating to the thickness of continental lithospheric plates and the

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An island arc section in the Himalayas

from Mike Coward, Geoffrey King and Brian Windley

THE newly-constructed Karakoram Highway in Pakistan provides access to (and excellent exposure of) the magnificent Kohistan island arc section. A recent conference of an International Geodynamics Working Group* provided much information on the geological and structural setting of the Kohistan island arc as well as a visit to the site.

At the conference Qasim Jan and K. Tahirkheli (University of Peshawar) and the French group from the Geology Department, Montpellier University described the structure cut by the Karakoram Highway in the Kohistan Himalayas. They interpreted this as a complete section of a late Cretaceous island arc, standing on end. At the base of the section, garnet and pyroxene rocks interpreted as mantle are overlain by amphibolite and metagabbro. Above this is a huge elongate layered metanorite lopolith at least 15 km thick and 300 km long. Above this and apparently related to it are calc-alkaline intermediate and acid intrusive and volcanic rocks topped by a magnificent section of pillow lavas and volcanic derived sediments.

This is the first complete section of continental style crust to be identified. It

*Held at the University of Peshawar, Pakistan on 19–27 November, 1979.

extent of under and overplating above destructive margins. Oxburgh argued that the continental lithosphere can be at most 250 km thick, a conclusion contested by T.H. Jordan (Scripps Institution, San Diego) who presented evidence that the lithosphere (tectosphere) beneath cratonic areas may be up to 400 km thick. However, this geologically reasonable model was strongly challenged by other geophysicists in the audience. Two contributions by H.D. Holland (Harvard University) and S.R. Taylor (Australian National University) respectively discussed the role of the atmosphere and hydrosphere and of sediments in crustal evolution; and J.V. Smith (University of Chicago) rounded off an interesting session with some speculations about the first 800 Ma of geological time (between 4,600 and 3,800 Ma ago).

The afternoon of the second day proved the most controversial part of the whole meeting. Chaired by the unashamedly 'accretionist' S. Moorbath (University of Oxford) it opened quietly enough with an exposition of the role of fluids, both magmatic and volatile, in crustal evolution (G.N. Hanson, State University of New York, Stony Brook). R.L. Armstrong (University of British Columbia) ably and entertainingly stated the 'conservationist' case, using evidence from a wide variety of sources, to support his thesis that the

is still apparently being emplaced as a massive thrust slab imbricated between the plate to the north carrying the Karakoram granite and thrust slabs of the Indian subcontinent. During the thrust process, which continued seismicity suggests is still active, the Kohistan sequence has been tilted so that the layering and bedding are vertical.

Future work on the exposed section will allow the chemical differentiation process in island arcs to be studied. Some aspects of the process are already obvious and interesting. Since the lopolith from which the higher level andesitic rocks derive is basic in composition, it seems that andesitic differentiation to form calc-alkaline rocks occurs at crustal levels rather than deeper in the mantle and the details of this can be studied.

Further studies of structure and seismicity should also elucidate the processes whereby dense material can be lifted by thrust processes to high crustal levels and may also indicate the depth at which decoupling occurs in lower lithosphere.

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continental crust has not grown significantly since early Precambrian times, but has been quantitatively recycled since it was first fully formed early in the Earth's history.

The discussion which followed showed this school of thought to be in a minority, but it also demonstrated that the difference between the two schools may be more imagined than real: few would dispute that there is some recycling of continental crust, the question seems to be simply: 'how much'. On the basis of some crustal modelling, R.K. O'Nions (University of Cambridge) argued that although most continental crust formed by about 3×10^9 years ago, the relationships between Nd, Sr, and Pb isotopes are consistent with a small amount of crustal recycling.

Finally D.P. McKenzie (University of Cambridge) focused on the geophysical constraints on models for the early Precambrian, when heat generation was much greater than now, and provided a salutary reminder that crust and lithosphere cannot be treated separately when discussing crustal evolution.

All in all, there was little if any 'crossing the floor' at this meeting, the protagonists seem to have gone their separate ways unconvinced by each other's counter-arguments. But this was a timely, entertaining and authoritative review of the contrasting lines of thought. □

REVIEW ARTICLE

Particle beam weapons—
a technical assessment

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Cambridge, Massachusetts 02139*This article explores the feasibility of particle beam weapons, identifies the physical principles that would govern their operation, assesses the technical difficulties, and evaluates their potential uses.*

THE US and the Soviet Union are thought to be working on the design of particle beam weapon systems¹⁻³. We describe here the physics of the generation, propagation, and interaction with a target of a beam of particles intense enough to inflict damage or destroy the target. We analyse the operational aspects of a particle beam weapon system: Who could use it and for what missions. How one could track the target and aim the beam with accuracy dictated by the zero-miss requirement inherent in this type of weapon. How the damage inflicted on a target could be assessed. How the entire weapon system could be commanded and controlled, and what countermeasures could be used against it. Laser beam weapons are not considered.

We examine the possibility of an energetic beam of particles as a weapon system with either exoatmospheric or endo-atmospheric applications. Based on a satellite orbiting about the Earth, the weapon could be used to shoot down intercontinental ballistic missile (ICBM) boosters in the powered-boost phase of their orbits, after they have ascended above the atmosphere. It could also be used to attack space-borne assets of another country. And, it could attack individual reentry vehicles (RVs) as they speed towards reentry into the atmosphere shortly before they reach their intended targets. An endoatmospheric particle beam based on the ground could defend point targets such as missile silos against reentering warheads or, if placed on a ship, could defend it against cruise missiles.

For use against ICBM boosters, the particle beam weapon could be deployed on an orbiting platform either in a near-Earth orbit of $\sim 1,000$ km above the Earth's surface, or in a geosynchronous orbit, at an altitude of $\sim 40,000$ km. From the latter orbit two satellites would suffice for continuous Earth-wide coverage of all possible ICBM or submarine launched ballistic missile (SLBM) launching sites. From a 1,000 km orbit, assuming horizon-to-horizon coverage, one would require a minimum⁴ of 29 platforms with particle beam weapons. A more realistic value for the angular coverage gives⁴ 120 as the number of platforms. In all cases, to be effective as an ABM system, each weapon would have to neutralise $\sim 1,000$ missiles during the first 5 min of their flight; that is, the system must be capable of destroying one target every 0.4 s. Unlike an antiballistic missile (ABM) system that uses missiles with nuclear warheads, a particle beam weapon requires a direct hit on the target to destroy it. As the characteristic length of an ICBM booster is of the order of 10 m, and the distance to it from the platform typically 1,000 km, the beam would have to be aimed with an accuracy of better than 10 p.p.m., that is, $\sim 1,000$ times more accurately than what an ordinary ABM system requires. Also, the system would need to be designed to fire several times per second at any one of the 1,000 targets, adjusting the aim each time to compensate for the miss distance of the previous shot.

Alternately, a particle beam weapon system based on orbiting platforms could be used to attack ballistic reentry vehicles that carry nuclear warheads to US targets. This approach for ballistic missile defence has no advantages over one designed to attack

the missile boosters. Reentry vehicles are considerably smaller than an ICBM booster, which complicates the problem of their detection, identification, and the aiming accuracy. Although they may be targetable from an orbiting platform for more than a few minutes, their large numbers (the Soviet Union is deploying missiles with 8–10 RVs per booster) require faster system response.

A third exoatmospheric use of a particle beam weapon would be as an anti-satellite system. When used for long-range applications against satellites in very high orbits above the Earth, the system would encounter the same problem of beam aiming and tracking as in the ABM use, except that in this case the target could be held under attack for a long period of time.

Endoatmospheric applications would include ground- or ship-based systems either for the point defence of silos against reentry vehicles, or of ships against cruise missiles. An ICBM RV on a normal trajectory would rise above the radar search horizon some 4,000 km, or ~ 10 min travel, away from the radar. For a cruise missile travelling near the surface at a speed of Mach 1, the radar detection distance could be as short as 1 km or about 4 s away.

To determine the performance parameters and physical characteristics of a particle beam weapon we began at the target, by first calculating the amount of energy that would damage a target, then examined the propagation of a particle beam through the atmosphere or the vacuum of outer space, and finally specified the characteristics the beam must have at the exit of the accelerator. On the basis of such considerations, one can calculate the required parameters of the accelerator.

Our assessments sometimes use idealised models of, often, very complex physical phenomena. However, our calculations tend to overestimate the effectiveness of the beam as a weapon.

Damage mechanisms and energy deposition on target

To determine the energy per unit area that the particle beam must deposit to be an effective weapon, one must consider the mechanisms by which deposition of this energy on a target would destroy it or cause it to malfunction. The mechanisms considered are: boring a hole in the outer shell of the ICBM or cruise missile, melting or damaging the guidance and/or control electronics of any of the targets; or exploding the high explosive trigger(s) of the nuclear warhead(s) carried by an ICBM or cruise missile.

A charged particle can cause chemical explosives to detonate by several cooperative mechanisms. Most chemical explosives will explode if their temperature is raised to $\sim 500^\circ\text{C}$ when confined. In the presence of the beam, this threshold temperature may well be lower, because of electric field and shock wave creation within the material. Thus, $\Delta T = 500^\circ\text{C}$ is a conservative figure. The energy density ϵ required to detonate the explosive is then given by $\epsilon = 3Rp\Delta T/M$ where R is the universal gas constant, and ρ and M are the mass density and

molecular weight of the explosive, respectively. Taking $\rho = 0.8 \text{ g cm}^{-3}$ and $M = 50$, yields $\epsilon = 250 \text{ J cm}^{-3}$. Similarly, knowing the specific heat, melting temperature and the heat of fusion of materials, the energy density necessary to melt them can be computed. To melt the aluminium shell of the weapon requires $\epsilon \approx 3,200 \text{ J cm}^{-3}$ and to melt the silicon substratum of electronic components requires $\epsilon \approx 7,000 \text{ J cm}^{-3}$.

Electronics, however, are not only sensitive to catastrophic failure due to an increase in temperature. Rate-dependent effects, caused by the creation of a large number of electron-hole pairs generated during the passage of a charged particle beam through the electronics, can also destroy the circuits. For example, recombination of the pairs could generate enough heat to damage circuit elements. The threshold for this effect in silicon is $\sim 10^{13} \text{ rad s}^{-1}$ or $2.4 \times 10^8 \text{ J cm}^{-3} \text{ s}^{-1}$. Thus, a beam that could deliver 10^3 J cm^{-3} in $1 \mu\text{s}$ could damage electronic circuits via this mechanism. Alternatively, production of ions which are trapped inside the microcircuits can form inversion layers which interrupt operation. Incident radiation can shift switching thresholds and cause source leakage in metallic oxide silicon circuits, which drains the power supply. Switching times can be affected: an energy deposition of $\sim 25 \text{ J cm}^{-3}$ would be enough to cause havoc in the guidance or control electronics of a missile or satellite. These results are summarised in Table 1.

Table 1 Estimated minimum deposition energy densities on various targets

Material	Type of damage	Energy density ϵ (J cm^{-3})
Aluminium	Melting	3,200
Chemical explosive	Detonation	250
Silicon	Melting	7,000
Silicon	Electron-hole recombination in circuits	1,000
Semiconductors	Shifts in circuit switching thresholds and times	25

The total energy E that must be deposited on target to cause damage is given by $E = \epsilon l A$ where ϵ is the appropriate energy density as given in Table 1; $l \equiv E \text{ dz/dE}$ is the scale length for energy absorption, that is the distance over which the beam particles are degraded in energy to $(1/e)$ th of their initial value, as a result of passage through the medium; A is the cross sectional area of the beam illuminating the target, and depends on the optics of the beam but not on target size.

A beam for a functional weapon seems to be one with $\epsilon \approx 1,000 \text{ J cm}^{-3}$ and $A = 10^4 \text{ cm}^2$. With $l \approx 9 \text{ cm}$, a value appropriate to the passage of an electron beam of several hundred MeV through aluminium, one obtains $E \approx 100 \text{ MJ}$. This then, is the energy that must be supplied by the accelerator. If V is the beam voltage, I the beam current, and τ the pulse length, we have that $E = VI\tau = 10^8 \text{ J}$. Setting $V = 10^9 \text{ V}$ and τ , say, equal to $100 \mu\text{s}$, gives $I = 1,000 \text{ A}$. Note that if the beam is to have a cross-sectional area $A = 10^4 \text{ cm}^2$ on a target $1,000 \text{ km}$ away, the required angular beam divergence is only 10^{-6} rad , which is a very demanding accelerator beam emittance.

Exoatmospheric propagation of a particle beam

We now examine three effects which impede the propagation of the charged particles in the vacuum of outer space, and then consider systems using neutral beams.

Electron and proton beams: The first effect is the radial spreading of a charged particle beam caused by Coulomb repulsion between like charges. Using relativistic particle dynamics, one finds that the beam radius a at a distance z from the source is

determined from the expression,

$$aF(\ln(a/a_0)^{1/2}) \approx (z/\beta\gamma)(I/I_A)^{1/2} \quad (1)$$

where a_0 is the initial beam radius and F is Dawson's integral⁵; $\beta = v/c$ with v as the axial particle velocity, and $\gamma = [1 - (v/c)^2]^{-1/2}$. I is beam current and I_A the Alfvén current defined as⁶

$$\left. \begin{aligned} I_A &= 4\pi\epsilon_0 m_0 c^3 \beta\gamma / e \\ &= 1.70 \times 10^4 \beta\gamma \text{ A for electrons} \\ &= 3.12 \times 10^7 \beta\gamma \text{ A for protons.} \end{aligned} \right\} \quad (2)$$

For a $1,000 \text{ A}$, 1 GeV electron beam having an initial radius of 1 cm , and striking a target at a distance $z = 10^3 \text{ km}$, equation (1) yields $a \approx 15 \text{ m}$. For an identical proton beam, $a \approx 1.8 \times 10^4 \text{ m}$. Therefore, while electron beams cannot be entirely ruled out as a weapon by this phenomenon alone, proton beams would obviously have no practical use.

The second effect which interferes with the use of charged particle beams in outer space is the deflection they suffer in the Earth's dipole magnetic field. In a uniform magnetic field \mathbf{B} , the radius of curvature⁷ $R = (m_0\beta c/eB)\gamma$. Hence a 1-GeV electron beam directed from a $1,000 \text{ km}$ orbit above the Earth towards a target $100\text{--}200 \text{ km}$ above the Earth's surface will have a radius of curvature in the geomagnetic field of $\sim 110 \text{ km}$. A corresponding proton beam will have a 190 km radius of curvature. Therefore, these beams will never reach the target $1,000 \text{ km}$ away. We show below that higher energies will be of no use.

Charged particle beams are deflected from their original direction by the geomagnetic field. The uncertainty ΔR in the position of a 1-GeV electron beam due to an uncertainty of 1% of the value of the geomagnetic field is 100 m at a $1,000 \text{ km}$ range, as obtained from the equation $\Delta R = R(\Delta B/B)$. This forbids the aiming of the beam. Another serious problem is that the geomagnetic field can be greatly and unpredictably disturbed by a nuclear explosion in the stratosphere.

The alternative is to use neutral particle beams for exo-atmospheric applications. We have considered neutron, γ -ray, and neutral hydrogen beams. The first two are unsuitable because the beam divergence is intolerably large. The neutral hydrogen beam is more promising.

Neutral hydrogen, H^0 , beams: A neutral hydrogen beam is generated from an H^- beam, that is, hydrogen atoms with one extra electron. Beams of these particles can be accelerated⁸ to $\sim 500\text{-MeV}$ energies in circular accelerators with a confining magnetic field of $\sim 10,000 \text{ G}$. The maximum energy achievable by this process is limited by the Lorentz force. That is, the particle feels an electric field of magnitude $q|\mathbf{v} \times \mathbf{B}|$ which causes distortion of its atomic potential and, if sufficiently large, leads to subsequent breakup of the atom. In a linear accelerator, because of relativistic effects, it is difficult to achieve energies $\geq 800 \text{ MeV}$.

The beam of H^- particles is passed through a system of magnetic lenses to focus and aim it. It is then neutralised by removing the extra electron by various methods such as passing it through a gas stripper, the Lorentz force generated by an externally imposed magnetic field, a plasma cell, or by ejecting one of the bound electrons with a laser beam. The gas-stripper approach seems to be the most direct. The cross-section for the stripping reaction is large (typically one-tenth of the geometric cross-section of the atom) and the efficiency^{9,10} of converting H^- to H^0 can be as high as 50% after passage through a few microgrammes of gas. To estimate the minimum divergence θ of the H^0 beam, we assume that the incident H^- beam has zero transverse momentum when incident on the gas stripper. From the uncertainty principle it is readily shown that $\theta \approx 2[(m_0/M)E_{\text{H}^-}/E]^{1/2}$ where m_0 is the electron mass, M the proton mass, E_{H^-} the binding energy ($\sim 1 \text{ eV}$) of the electron in the H^- ion, and E the energy of the beam. Setting $E = 500 \text{ MeV}$, gives $\theta \approx 2 \times 10^{-6} \text{ rad}$, a value which is sufficiently small to make the neutral hydrogen beam of potential weapon interest.

There are, however, several problems. First, the beam divergence calculated above represents a lower limit as it ignores the divergence of the incident H^- beam; the total angular spread due to the addition of spreads from two (independent) sources is given by $(\theta_0^2 + \theta^2)^{1/2}$ where θ_0 is the spread of the incident H^- beam. Therefore, it is not certain that accelerator technology will make it possible to construct an accelerator having the necessary small emittance, large beam current, and large beam energy. Second, if the beam size is to be maintained, the magnetic lens that bends the beam must be achromatic to 1% or better and have a stable and predictable field strength to 1 p.p.m., requiring that the system of magnets be carefully shielded from the geomagnetic field. And third, the determination of the miss vector is very difficult with a neutral beam.

One can also generate a neutral beam through the capture of electrons by a beam of positive ions, leading to the formation of "plasmoids". However, such beams have large beam divergencies and are not suitable for weapon use.

Endoatmospheric propagation of a particle beam

When a charged particle beam is fired into the atmosphere, it partially ionises the gas in its immediate neighbourhood. The plasma thus generated helps to reduce the radial electric field associated with the primary beam, to the point that it is balanced by the beam's self-magnetic field. In principle, then, it is possible to propagate a charged particle beam in the atmosphere. In the following we examine its propagation characteristics.

Beam propagation: The magnitude of the allowed beam current I is constrained by the fact that as the current in, say, a cylindrical beam increases, so does its self-generated azimuthal magnetic field B_θ . An individual beam electron subjected to this field will undergo radial oscillations, and if I and thus B_θ are large enough, it will be deflected backwards⁶, opposite to its initial (axial) propagation direction. When the beam is completely space charge neutralised by the presence of the ambient ionised gas, and if moreover the beam has a uniform density over its cross-sectional area, one then finds⁶ that the maximum allowable current I_{\max} is the Alfvén current I_A given by equation (2). However, by proper beam shaping^{11,12} (for example, using a hollow, annular beam) I_{\max} can substantially exceed I_A . When a uniform beam is partially space charge neutralised, with a neutralisation factor f ($0 \leq f \leq 1$), the appropriate maximum current is now given by¹³

$$I_{\max} = 17,000 \beta^3 \gamma / (\beta^2 + f - 1) \text{ A} \quad (\text{for electrons}) \quad (3)$$

Equations (2) and (3) refer to infinitely long beams. However, one needs to consider the beam front where a rising current occurs. The time varying current generates a time varying azimuthal magnetic field $B_\theta(t)$ which in turn produces a time varying electric field $E_z(t)$ that drives a counterstreaming current in the ambient plasma. This is referred to as 'current' or 'magnetic neutralisation'. When magnetic neutralisation is complete (that is, the forward primary beam current equals the induced counterstreaming current) I_{\max} is unlimited in magnitude. If, however, magnetic neutralisation is only partial, one finds that,

$$I_{\max} = 17,000 \beta \gamma / (1 - f_m) \text{ A} \quad (\text{for } f = 1) \quad (4)$$

where f_m is the fractional magnetic neutralisation factor, $0 < f_m < 1$.

Knowledge of the magnitudes of f and f_m and of beam propagation characteristics in general requires detailed knowledge of ionisation cross-sections of atmospheric constituents and of beam dynamics. Partial (or complete) space-charge neutralisation f comes about through two processes: by direct ionisation of the gas by the beam, and by avalanching in the induced electric field¹⁴. In addition to these 'classical' processes, and at pressures below ~ 1 torr, ionisation by microinstabilities is prevalent. Here electron generation occurs as a result of the

strong turbulent electric field associated with the instabilities^{15,16}.

A typical experimental value of the ratio n_p/n_b of the secondary plasma density to primary beam density is, $10^3 \leq n_p/n_b \leq 10^4$. For example, when the beam current density $J_b = 1 \text{ kA cm}^{-2}$, $n_b = J_b/ec = 2 \times 10^{11} \text{ cm}^{-3}$ and it follows that $2 \times 10^{14} \leq n_p \leq 2 \times 10^{15} \text{ cm}^{-3}$. As relevant gas pressures lie in the range 4×10^{16} to $3 \times 10^{19} \text{ atom cm}^{-3}$ (atmospheric pressure), we see that the ambient gas becomes weakly ionised (degree of ionisation $\leq 2\%$), and the beam forms an electrically conducting channel of conductivity σ , where

$$\sigma = n_p e^2 / m \nu \quad (\text{S m}^{-1}) \quad (5)$$

Here ν is the electron-atom collision frequency for momentum transfer (for air¹⁷ $\nu = 1.8 \times 10^{-7} n_g \text{ s}^{-1}$ where n_g is the gas atom density in cm^{-3}). At $p = 2 \text{ Torr}$, $n_g = 7 \times 10^{16} \text{ atom cm}^{-3}$, and setting $n_p = 2 \times 10^{14} \text{ cm}^{-3}$, yields $\sigma = 460 \text{ S m}^{-1}$.

Magnetic neutralisation f_m becomes possible¹⁸ when the conductivity σ is large enough, enabling charge of the ambient plasma to move in the axial electric field E_z generated by the propagating electron beam. This electric field drives the return current I_r , whose current density is $J_r = \sigma E_z$. The magnetic neutralisation factor $f_m \equiv J_r/J_b$ is then given by $f_m = \sigma E_z/J_b = \sigma V/J_b L$ where V is the axial voltage drop along the beam, and L is its length. Setting $f_m = 1$, $\sigma = 460 \text{ S m}^{-1}$ and $J_b = 10^7 \text{ A m}^{-2}$, yields an axial electric field $E_z = 2.2 \times 10^4 \text{ V m}^{-1}$. We observe that the axial electric field causes an energy degradation of the primary beam. In going 1 km, each beam electron loses 22 MeV, a 2% energy loss from a 1 GeV beam. This, of course, is not the only energy loss¹⁹ by the beam particles.

Table 2 summarises qualitatively the propagation characteristics¹⁸ of an electron beam. Much experimental and theoretical work is needed for a clear understanding of this very complex problem.

Table 2 Propagation characteristics¹⁸ of an electron beam in air*

Air pressure (torr)	Typical value of f	Typical value of f_m	Beam behaviour
$< 10^{-3}$	≈ 0	≈ 0	Beam blows up
$\approx 10^{-1}$	≈ 1	$\ll 1$	Beam pinches
≈ 1	1	≈ 1	Beam drifts in a force-free environment
> 20	1	≈ 0	Beam pinches and then breaks up

* $V \approx 3 \text{ MeV}$, $I_b \approx 50 \text{ kA}$, pulse length $\approx 30 \text{ ns}$

Beam dispersion: Consider first an electron beam. To determine the increase in area along the beam axis caused by scattering, we assume that the beam is fully space-charge neutralised and that the ratio of beam current, I_b , to the Alfvén current, I_A , is $\ll 1$. This corresponds to the paraxial ray approximation for which the transverse particle velocity is small compared with the longitudinal velocity. The beam current density and the fields are taken to be azimuthally symmetric, with no density variations occurring across a transverse segment of the beam. The energy of the beam particles is assumed to remain constant, independent of distance. Only small-angle multiple scattering is considered, beam segments do not overtake one another in the direction of propagation, and effects of return current are neglected. Particles interact with each other by the collective fields they induce. All relevant time scales are long compared with the period of particle oscillation. In these conditions one obtains a form of Nordsieck's equation^{20,21} given by

$$(I_b/I_A) \ln [R^2(z)/R_0^2] = \langle \theta_s^2(z) \rangle - \langle \theta_0^2 \rangle + O(d^2 R^2/dz^2) \quad (6)$$

where I_b is the beam current and I_A the Alfvén current given by equation (2). $R(z)$ is the beam radius at some axial distance z , and R_0 is the beam radius at the injection point $z = 0$. The quantity $\langle \theta_s^2(z) \rangle$ is the mean square scattering angle due to scattering by the background gas and $\langle \theta_0^2 \rangle$ is the mean square

angle of the random component of the particle motion at the point of injection, $z = 0$. It is related to the accelerator emittance ϵ through²⁰

$$\langle \theta_0^2 \rangle = (\epsilon/R_0)^2 \approx (I_b/I_A) \ll 1 \quad (7)$$

For small beam spreading, the term $O(d^2 R^2/dz^2)$ appearing in equation (6) can be neglected.

The mean square scattering angle is given by²²

$$\delta \langle \theta_s^2 \rangle / \delta z = c_1 \ln(c_2 z^{1/2}) \quad \left. \begin{aligned} c_1 &= (8\pi e^4 Z(Z+1)n) / (m^2 c^4 \gamma^2 \beta^4) \quad \text{cm}^{-1} \\ c_2 &= (hZ^{2/3} n^{1/2}) / (\pi^{1/2} m c \beta) \quad \text{cm}^{-1/2} \end{aligned} \right\} \quad (8)$$

where Z is the atomic number of the gas (equal to ~ 7.4 for air) and n is the density of nuclei (equal to $2.69 \times 10^{19} \text{ cm}^{-3}$ at sea level). Integrating equation (8) with respect to z , and inserting the result in equation (6) yields

$$R^2(z)/R_0^2 = A(z)/A_0 = \exp [I_A/I_b (c_1 z \{ \ln [c_2 z^{1/2}] - 1/2 \})] \quad (9)$$

where $A(z)$ is the cross-sectional area of the beam at position z and A_0 is its area at the accelerator, $z = 0$.

The larger the beam current (consistent with $I_b/I_A \ll 1$) the smaller the beam dispersion. We take as an example our model beam: beam voltage = 1 GeV, beam current = 1,000 A. Then, at a distance z of 1 km, equation (9) gives $A(z=1 \text{ km})/A_0 = 7.0 \times 10^7$. A beam which is initially, say, 1 cm in radius expands to 84 m in a distance of 1 km. Note, however, that this is an optimistic lower limit on the dispersion suffered by the beam. For beams with energies ≥ 100 MeV, the principal energy loss¹⁹ is through bremsstrahlung. The beam energy degrades rapidly as a result of this loss, and if E_0 is its initial energy, then at a distance z , $E(z) = E_0 \exp(-z/z_0)$ where for air the 'radiation length' $z_0 \approx 300$ m. As a result, the scattering increases since in accordance with equation (8), $\delta \langle \theta_s^2 \rangle / \delta z$ varies as $[E(z)]^{-2}$. Thus, even in a distance z as short as one radiation length, the electron beam disperses hopelessly.

Now consider a proton beam with the same specifications of energy and current (1 GeV, 1,000 A). Blanchard²³ obtains for the mean scattering for heavy particle incident on matter,

$$\langle \theta_s^2 \rangle = \langle \theta_0^2 \rangle + 2\kappa Z(m_e/m_p) \ln \left[\frac{E_0(E + 2m_p c^2)}{E(E_0 + 2m_p c^2)} \right] \quad [10]$$

where E_0 and E are the proton kinetic energies at the beginning and the end of the path, respectively, and κ is a coefficient of the order of 1 (≈ 0.75 for air), having a weak dependence on the nature of the ambient medium and on the proton energy. Using the results of Bethe and Ashkin²⁴ for proton energy loss due to both hard and soft collisions, one finds, for protons with an initial energy $E_0 = 1$ GeV, a loss of ~ 130 MeV in traversing 1 km of atmosphere. Hence $E = 870$ MeV and equation (10) yields $\langle \theta_s^2 \rangle \approx \langle \theta_0^2 \rangle + (5.5 \times 10^{-4})$. Substituting this result in equation (6) and noting that for protons $I_A = 3.12 \times 10^7 \beta \gamma A$ (see equation (2)), it follows that $A(z=1 \text{ km})/A_0 \approx 10^{13}$. Beam dispersion is immense. Indeed, A/A_0 is so large that equation (6) from which this ratio was derived has long ceased to be valid. What actually happens is that the protons do not propagate as a beam but form a cloud of positive charge near the outlet of the accelerator. In addition, above 1 GeV, endoatmospheric propagation of protons is limited primarily by nuclear collision which results in a particle number loss from the beam.

Hole-boring: The above results indicate that electron or proton beams are not suitable for endoatmospheric transport of energy over any distances useful for a weapon. An alternate approach is to attempt to bore an evacuated channel through the intervening atmosphere to the target through which a particle beam could propagate. This reduces the density n of scatterers and thus the scattering angle θ_s (see equation (8)).

To calculate the amount of energy that will be removed from the beam to bore a hole, consider a pulsed 1-kA electron beam of 1-GeV energy. The particles will lose energy by two effects: ionisation and bremsstrahlung. For air at STP, the ionisation loss

is²⁵ $\sim 2 \times 10^3 \text{ eV cm}^{-1}$ for energies of ~ 1 GeV. The ratio of radiation loss to collisional loss is determined by the Z of the scattering material and the energy of the beam, and is roughly $EZ/1600 m_0 c^2 \approx 9.1$.

Take a beam with an area $A_0 = 1 \text{ cm}^2$ at the accelerator port and an initial current I_b of 1.0 kA. The beam will then deposit energy by ionisation at a rate of $2 \times 10^6 \text{ W cm}^{-3}$ and by bremsstrahlung (mainly in the forward direction) at a rate of $1.8 \times 10^7 \text{ W cm}^{-3}$. Both of these effects will contribute to superheating of the air in the region of the beam, and the consequent formation of a channel of reduced density through which successive pulses could pass. This is the concept of hole-boring. To reduce the gas density in the channel to, say, $0.1 \times$ atmospheric density, the temperature must rise to $\sim 3,000^\circ\text{C}$. For a channel of 1 cm^2 cross-sectional area and 1 km long, the beam must deposit an energy of $\sim 1.5 \times 10^6 \text{ J}$.

By reducing the air density in the channel from atmospheric to $0.1 \times$ atmospheric, the radiation length for bremsstrahlung loss by electrons increases from ~ 300 to $\sim 3,000$ m. Thus, in propagating 1 km, energy loss can be neglected, and γ of equation (8) remains essentially constant. Beam dispersion is calculated in the same way as was done in the previous subsection. For a 1-GeV, 1-kA electron beam one finds that $A/A_0 = 4.4$, which is an acceptably small spreading for weapon use.

Hole boring is indeed necessary for stable beam propagation. Table 2 and experiments^{14,26} indicate that there is a range of pressures within which stable electron beam propagation may exist. For air at room temperature the 'pressure window' extends from ~ 1 to 10 torr. The good propagation characteristics are interpreted²⁷ as a combination of two effects: the quenching of a two-stream instability by sufficiently high plasma density and electron-atom collisions; and the simultaneous suppression of the resistive hose instability caused by sufficiently high electrical conductivity generated by the ionisation processes described earlier.

Endoatmospheric electron beam propagation might, therefore, be an interesting candidate as a weapon, provided that a hole can be bored through the atmosphere that would have a neutral density n around 0.3 to $3 \times 10^{17} \text{ cm}^{-3}$. The crucial question is will the beam remain in the channel over distances of, say, 1 km? Reported experimental work^{14,26} on this problem involves laboratory measurements over propagation distances less than several metres, and with beams having energies of the order of 10 MeV. Little is known about electron beams with energies in the GeV region.

Accelerator characteristics

There are two types of accelerators in existence: (1) conventional high voltage, low current machines capable of delivering high quality beams of modest energy per pulse; and (2) accelerators based on novel technology^{28,29} developed during the 1960s. The latter are characterised by relatively low voltages, but high currents and exceedingly large beam energies. The properties of the two types of accelerators are summarised in Table 3, together with a list of parameters desirable in an accelerator meant for a particle beam weapons system: the parameters of existing machines are far removed from the needs of a weapon's type of generator.

An exoatmospheric particle beam weapon requires a machine that would accelerate a beam of H^+ atoms to about 1,000 MeV. H^+ ion beams are routinely accelerated in proton linear accelerator systems at the Argonne National Laboratory and at the Los Alamos Meson Physics Facility. Recent developments in ion source technology, both in the US and the USSR⁶, indicate that high-current/low-emittance H^+ atom beams are possible, with currents of ~ 0.15 A, and a beam divergence of $\sim 20 \mu\text{rad}$. As the current is at least three orders of magnitude less than that of what a beam weapon would require, technological developments in the near future are unlikely to achieve the requisite currents (~ 1 kA) and beam divergence ($\sim 1 \mu\text{rad}$) for a beam weapon accelerator.

The electrostatic acceleration of the beam to adequate energy for injection into a r.f. linac is done by several different accelerator types, such as a van de Graaf, and offers no technological problem. The main section of the r.f. linac, however, is a problem. None of the existing machines has all of the properties necessary for exoatmospheric particle beam weapon applications. For instance, the BNL and the Fermi Lab injector linacs both accelerate 0.15–2 A currents in short pulses. Moreover, existing proton linac accelerating gradients are low, $\sim 2 \text{ MeV m}^{-1}$. Experience with electron linear accelerators indicates that gradients of up to $\sim 30 \text{ MeV m}^{-1}$ may be sustained. This makes for extremely long machines when $\sim 1 \text{ GeV}$ beams are desired.

Table 3 Characteristics of the two types of large accelerators, and the desired characteristics of an accelerator for use with particle weapons

Parameter	High-voltage, low-current accelerator	Low-voltage, high-current accelerator	Particle-beam weapon accelerator
Voltage (MeV)	1500	15	1,000
Current (A)	0.025	10^5	1,000
Pulse length (μs)	1.5	0.12	100
Energy per pulse (J)	56	1.8×10^5	10^8
Pulse repetition rate	50 pps	Several per day	≥ 100 pps
Beam divergence (rad)	$\geq 10^{-5}$	$\geq 10^{-2}$	$\sim 10^{-6*}$

* The quoted value of $1 \mu\text{rad}$ is for exoatmospheric weapons systems only; for endoatmospheric use, much poorer emittances would suffice.

Providing adequate power for an accelerator in space should not pose serious technological problems. The detonation of explosives coupled into appropriate generators could provide the power required. A device for staging the energy to the time scale required for an accelerator, with the capacity to store enough energy with easy retrieval at high efficiency and in a short time, would be necessary. Such staging devices are not apparently beyond current technological capabilities. If we assume an (optimistic) energy conversion efficiency from staging system to beam energy via the accelerator of 50%, and an energy conversion efficiency from primary power to the staging system of 30%, the primary power required will be about six times more than the beam power. Therefore, an accelerator that produces a 100-MJ beam (see Table 3) will require 600 MJ of primary energy per pulse. The energy stored in high explosives is $\sim 4 \times 10^3 \text{ J g}^{-1}$, and it follows that the requisite primary energy can be supplied by the explosion of $\sim 150 \text{ kg}$ of high explosives per pulse.

Another type of accelerator, the linear induction accelerator, is capable of short pulses of very high current and has been developed for various applications. A typical linear induction accelerator is the ERA 4-MeV induction linac built in Berkeley. The accelerator produced a current of 1,200 A for 40 ns at an output energy of 1–4 MeV. The system is capable of reproducible high current operation; it is, however, bulky and heavy as it consists of iron or ferrite cores for the transformer coupling. In the Berkeley ERA injector case the energy gradient was 0.73 MeV m^{-1} . Presumably this gradient can be increased to about twice this value, but a 1-GeV accelerator will be at least 400–500 m long, with current technology. For a 1,000 section accelerator the weight of iron required is about 100 tons, a reasonable value. The electron gun system necessary for this accelerator is available, and the emittance is suitable for a small diameter output beam.

The unresolved technical questions are whether the energy gradient in such machines can be increased to the point that the overall size of the accelerator becomes practical, and whether a

few kilohertz pulse rate that seems essential for useful operations can be achieved.

Operational requirements of a particle beam weapon system

The operational requirements that various missions dictate are:

- Detect and track the target(s) with appropriate sensors.
- Identify the target(s) among decoys.
- Point the beam at a target and follow the target.
- Fire the beam towards the target.
- Determine if the target was hit, or not.
- Assess the damage if the target was hit.
- Determine the miss vector if the target was not hit.
- Correct the aiming of the beam by the miss amount.
- Fire the accelerator again.
- Determine the miss distance or assess the damage to the target once again.
- Repeat this cycle until the target is verifiably destroyed.
- Communicate the results to a command center.
- Repeat the cycle with the newly engaged next target.

We consider below the performance requirements of the system in several conditions.

Antisatellite, space-based system: In this case the target object would have to be detected by long-range radar and its position further refined by means of an optical sensor. Since the target is typically a few metres in diameter, the weapon system would require optics with at least a 50-cm mirror to resolve the object 1,000 km away. If the target object is $\sim 40,000 \text{ km}$ away, the mirror would have to be 20 m. Even then, the weapon system could not readily assess either the damage inflicted on the target or the miss vector if the target were not hit, and therefore the weapon's operator has no way of knowing whether or where to re-aim the beam for a second burst. He has two choices: either fire once in a 'blind' fashion and assume that the target is impaired, or fire repeatedly until the target satellite tumbles or explodes.

Antiballistic, space-based system: A space-based boost-phase ballistic missile defence particle beam system would have to have similar subsystems as above for target detection and tracking, beam aiming and damage assessment, but the time constants of this operation are very different from an A-SAT mission. A single particle beam weapon must be capable of detecting, identifying, and tracking as many as 1,000 boosters while still in their boost phase, that is, for at most 400 s. The tracking and aiming mechanism must track each booster with an accuracy of one part in 10^5 . The aiming mechanism for a neutral beam could consist of a precalibrated magnetic lens and of focusing magnets which can be mechanically steered for accurate aiming by means of an optical telescope. This would require an achromaticity of the magnetic system of 10^{-6} so that the beam divergence could be maintained at a minimum. As the variations of the geomagnetic field are not known to such accuracy, the entire magnet complex would have to be magnetically shielded. It is very difficult to ascertain the size of the miss vector between a neutral beam and the target, and it may be necessary to fire repeatedly but blindly at a target until there are visible catastrophic results: the ICBM either explodes or tumbles out of control. An alternative to this lengthy process is to preprogram the weapon to fire stochastically a fixed number of particle pulses at a target and then shift to another one without positive identification of a successful hit.

One may also contemplate tracking the H^0 beam with a laser that would induce resonance fluorescence³⁰ (resonant Rayleigh scattering) in the hydrogen atoms. This would necessitate the use of an as yet unavailable, pulsed, tuneable laser emitting $\sim 1 \text{ J}$ of energy in the $1,000 \text{ \AA}$ wavelength range. The number n of fluorescent photons backscattered into the receiving-transmitting mirror of area A situated at a distance R from the scattering volume V is given by

$$n = \left(\frac{E}{h\omega} \right) \left(\frac{A}{A_p} \right) \left(\frac{r_0}{R} \right)^2 \left(\frac{\omega}{\Delta\omega} \right)^2 N_H V \quad (11)$$

Here E is the laser energy and ω is its frequency; $\Delta\omega$ is the line width of the laser, or of the resonance H^0 transition, whichever is the larger; $r_0 = e^2/4\pi\epsilon_0 m_e c^2$ is the classical electron radius, A_s the cross-sectional area of the scattering volume V , and N_H is the number of density of H^0 atoms. Taking $E = 1\text{ J}$, $\hbar\omega = 10\text{ eV}$ ($\lambda = 1,240\text{ \AA}$), $R = 10^3\text{ km}$, $V = 100\text{ m}^3$, $A/A_s = 0.018$, $\omega/\Delta\omega = 1.1 \times 10^7$, and $N_H = 2.1 \times 10^{13}\text{ m}^{-3}$ (which corresponds to a 1 kA beam of 1 m^2 cross-sectional area), yields $n = 2.3 \times 10^4$, which suffices for detection purposes. Note that in obtaining this value, we chose a diffraction limited beam divergence of 10^{-6} rad and an (optimistic) line width $\Delta\omega = 2\omega^2 r_0/3c$ equal to natural line broadening.

To track the H^0 beam and determine its position requires the laser to have a much greater pulse repetition rate than that (≥ 100 pulses per s) of the hydrogen beam. Knowing the direction of the beam axis does not resolve the problem of target tracking and aiming discussed above.

We conclude that even if an H^- atom accelerator and stripper system that would produce a neutral beam suitable as a lethal mechanism could be built, it would still be technically infeasible to deploy a particle beam weapon system that could be even marginally effective against a massive ICBM attack on the US. It is unreasonable to assume that a boost-phase ballistic missile defence system would operate in a cooperative environment, as it would have to operate mostly within line of sight of enemy territory. We must consider the countermeasures that a military commander could take against the threat of a particle beam space-borne ballistic missile system. The most obvious method would be to destroy or mine the particle beam accelerator. Weapons based in space during peace time are very vulnerable to small explosive charges on small satellites which have been placed in orbit very close to them.

The operations of a space-based particle beam weapon system would probably be closely controlled from the ground. Interfering with the link between the weapon platform and its ground control would effectively nullify the weapon. Another countermeasure is to deny the system knowledge of the precise location of its targets. This could be done either by jamming the radar which the system must use for initial detection and localisation of its targets, or by the use of offset decoys for radar, visible, or IR. For space targets one can use a chaff shroud or umbrella so that even if the particle beam system could detect the general location of the target by radar, it could not see through the shroud. As the chaff or thin aluminium sheets can be carried by the ICBM booster, such countermeasures would be effective in the case of sea-launched as well as land-based ICBMs.

Another effective countermeasure against a neutral hydrogen atom beam weapon based in space is to interpose a thin layer of air between it and the ICBMs that it is designed to attack during their boost phase. The atoms in the neutral hydrogen beam will be stripped of their electrons and become protons. As indicated above, protons of that energy cannot propagate in space as a beam because they disperse rapidly. The stripping cross-section of a 1-MeV hydrogen atom incident on a nitrogen molecules is $\sim 7 \times 10^{-17}\text{ cm}^2$ (refs. 31, 32). The cross-section falls off roughly logarithmically with increasing energy, and we estimate that at 1-GeV the cross-section is $\sim 10^{-17}\text{ cm}^2$. Now consider filling a volume $4 \times 10^{24}\text{ cm}^3$, an area of $2,000 \times 2,000\text{ km}$ and $1,000\text{ km}$ high, with sufficient air molecules to cause stripping. Using the above value of the cross-section, one finds that the mass of air required is $5.5 \times 10^5\text{ tons}$, and the energy necessary to lift it to the requisite height is $2.7 \times 10^{15}\text{ J}$. As 1 g of high explosives has $\sim 4 \times 10^3\text{ J}$, an explosion of a 0.7 Mton nuclear warhead could provide the energy necessary to raise the layer of air that would disperse the particle beam.

Ship-based, counter-cruise missile system: This mission includes the same operations outlined for the exoatmospheric weapon, except that the command and control systems will be integrated into the ship-based weapon and therefore the communications link cannot be interfered with. Another difference is that the counter-cruise missile weapon has only $\sim 3\text{ s}$ to engage and

destroy its target if it starts its attack at 1 km range, and that the number of targets at any instant would be fewer than in the ABM case, perhaps no more than three or four.

If the weapon does not shoot down the missile with the first beam burst, it must track the target and shoot again. It is not clear, however, that the beam can track a target that has any significant angular velocity with respect to the ship. To accomplish that, the beam would have to leave the evacuated channel that it propagated in and bore another very near the previous one. It is not known whether the atmosphere can support stably such a procedure or how this procedure depends on the angular velocity of the target, the size of the channel, the state of the atmosphere in the immediate vicinity of the ship, or how reproducible such behavior could be.

If the attacking cruise missile can manoeuvre in response to an attack by a charged particle beam, an erratic course after a beam burst would not be a definite indication of decisive damage. A certain kill against a cruise missile would be signalled by the explosion of its warhead or the plunging of the missile into the water. The missile then must be fired at until it is killed and that requires tracking capability for the beam. A missile armed with a nuclear warhead can destroy a ship even if it explodes some distance from it, and that may shorten the time available to the defence to destroy it.

The attacker can also use several countermeasures against a ship-borne charged particle beam weapon. For example, a transonic cruise missile without power and a ratio of lift to drag of 7 loses only 2% in velocity in the first 1 km of glide. In these conditions, multiple decoys could be popped out of a cruise missile, all of which would glide towards the target for the last 1 or 2 km , confusing the defence. Cruise missiles attacking a ship could use an ahead-fired smoke rocket to impair tracking by optical systems, or could fire ahead either chaff for countering radars, or even small explosive rockets for disturbing the uniformity of the air ahead, if strict uniformity is required for charged particle beam propagation.

A land-based ABM system using a charged particle beam as a kill mechanism suffers from the same basic vulnerabilities as earlier ABM schemes^{33,34} that utilised missiles as RV interceptors. The fact that a charged particle beam moves very nearly at the speed of light while missile interceptors are only hypersonic, does not remedy these vulnerabilities.

Conclusions

The current state of accelerator technology is reflected in Table 3. There is no machine which combines the high energy, high current, beam achromaticity and normalised emittance required of an accelerator suitable for a weapon system. This does not mean that accelerators with performance characteristics required for a beam weapon system could not be built. But the operational difficulties of a particle beam weapon outlined here seem insurmountable.

The cost-effectiveness of a charged particle beam system must also be compared to alternate systems, as for example, high energy lasers. Exoatmospheric uses of lasers apparently offer a more near-term operational capability, and also involve a much more available technology that one can assess and evaluate. For endoatmospheric uses, charged particle weapons are faced with competing conventional weapons such as guided projectiles, hypersonic missiles, and rapidly firing point-defence guns. Therefore, in an environment of finite resources for military research and development, it seems unwise and wasteful to develop particle beam weapons, before a detailed systems analysis of countermeasures is made. The performance characteristics of such a system do not overcome the vulnerabilities and weaknesses of earlier ABM schemes^{33,34} that used missiles as interceptors, nor do they lend themselves to the development of an effective substitute of these earlier ABM systems.

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ARTICLES

Fission track age of the KBS Tuff and associated hominid remains in northern Kenya

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Fission track ages of pumice from the KBS Tuff have been redetermined using carefully controlled etching and counting procedures. Primary igneous zircons from five pumice samples give a mean age of 1.87 ± 0.04 Myr. The presence of detrital minerals of two different age groups has important implications for the controversy over the age of this tuff.

OVER the past 10 yr the Plio-Pleistocene sedimentary basin on the eastern shore of Lake Turkana (formerly Rudolf) in northern Kenya has been the site of an intensive study of early man and his contemporary environment. Hominid remains, other vertebrate fossils and archaeological material have all been recovered in abundance from this area and various aspects of this multidisciplinary research effort have been described recently¹⁻³.

It was realised very early that several tuffaceous horizons occurring within the sedimentary succession could be used to establish a series of radiometrically dated marker horizons⁴⁻⁷. Deposition of these tuffs in their present locations is thought to have occurred rapidly after the eruptions which produced them. Measured ages on the igneous materials can, therefore, be related directly to the stratigraphic ages of the surrounding sediments and the fossils they contain. Figure 1 gives a generalised stratigraphic column showing the relative positions of the major tuffaceous horizons in the Koobi Fora Formation of the East Turkana Basin. Also shown are the approximate positions of a few of the most important hominid and archaeological sites and their relationship to the KBS Tuff.

Results of K-Ar, ⁴⁰Ar-³⁹Ar and fission track dating studies on these tuffs have been controversial and particular problems have been associated with the KBS Tuff. Fitch and Miller⁸ originally dated feldspars from pumice in this tuff at 2.61 ± 0.26 Myr using the ⁴⁰Ar-³⁹Ar method and although further work^{9,10} produced a scatter of ages from 0.52 to 2.64 Myr this remained their preferred age. Curtis *et al.*¹¹, however, obtained mean conventional K-Ar ages of 1.82 ± 0.04 Myr and 1.60 ± 0.05 Myr respectively for tuffs mapped as the KBS in two different areas of the East Turkana Basin. Subsequently Fitch *et al.*^{12,13} revised their former estimate to 2.42 ± 0.01 Myr in apparent agreement with an average zircon fission track age of 2.44 ± 0.08 Myr reported by Hurford *et al.*¹⁴.

Note that the zircon fission track ages of Hurford *et al.* represent the first attempt at dating young zircons of this kind. These ages apparently agree closely with each other but this is

mainly due to the close communication between these authors on track identification and discrimination in these samples. Further work has not supported this consistency but initially produced a much larger scatter of results. It has become clear that this scatter was due to analytical rather than geological factors and that some of the potential difficulties had not been given adequate consideration in the earlier results.

The present study was initiated to overcome this unsatisfactory state of affairs by first establishing reliable fission track ages for zircons from the KBS Tuff. An important component in this project has been the development of improved techniques to overcome the particular problems that arise when dating zircons from geologically young rocks. The ultimate aim is to establish an independent fission track chronology for many of the tuffs in the East Turkana sedimentary basin.

Sampling and methods

None of the so-called tuffs in the East Turkana Basin represents primary pyroclastic deposits. All have been reworked by running water and redeposited as highly tuffaceous sediments. The deposits are not pure volcanic material and have been shown to contain varying amounts of detrital minerals⁷ which could seriously affect attempts at dating. Sampling was therefore restricted to the occasional blocks of pumice (up to ~25 cm across) which are found in some of the tuffs as these represent relatively uncontaminated igneous material. Pumice samples were collected from the KBS Tuff in Fossil Collection Areas 105, 112 and 131 which are shown in Fig. 2. The detailed petrography and mineralogy of some of these samples has been described elsewhere¹⁵. The pumice from Areas 105 and 131 was found to contain small accessory zircon crystals (up to ~450 × 150 μm but mostly much smaller) suitable for fission track dating. No primary igneous zircons were found in pumice from Area 112.

One of the zircon separates originally dated by Hurford *et al.* (FMA517) was reanalysed in this study. These zircons were extracted from a sample of several pumice cobbles by dissolving

the crushed pumice in a mixture of HF and H₂SO₄ and recovering the crystals from the insoluble residue¹⁴. Zircons were extracted from two large single pumice blocks, FMA559 and FMA560, in essentially the same way. A different separation procedure was used for the two remaining samples (7722-numbers) both of which were composite samples of a large number of pumice cobbles. After an initial coarse crushing to <2 mm a panning procedure was used to separate crystals from the pumice. Zircons were then extracted from the phenocryst concentrate using conventional heavy liquid and magnetic techniques and an additional fraction was obtained by acid dissolution of the glass. Two other minerals suitable for fission track dating, apatite and sphene, were also recovered from the pumices but these were found to be almost entirely of detrital origin.

Fission track dating methods for zircons were based on those described by Gleadow *et al.*¹⁶ and the external detector approach for measuring induced tracks was used. Because of the very young geological age of these zircons, particular difficulties arise which are not generally encountered with older materials. As most previous experience with dating zircons has been with much older rocks than those studied here, these difficulties and their potential effect on the resulting ages have not been clearly understood. Some of the factors involved are as follows:

- (1) Very small numbers of fossil fission tracks are found in the zircons. This is exaggerated in the present samples by a much lower than average uranium concentration and often small grain size. The lack of tracks makes it difficult to assess the correct etching time for these zircons.
- (2) The zircons have a very low track etching rate due to the low level of α -radiation damage¹⁶ making it difficult to reveal all the tracks by etching. Also, the zircon grains tend to fall out of their FEP Teflon mounts during the long etching times required.
- (3) Etching of tracks lying in different orientations is highly anisotropic giving rise to etch pits of differing appearance and making identification more difficult.
- (4) Tiny acicular inclusions are present in the zircons from the KBS Tuff which are often of very similar dimensions to etched tracks and can easily be mistaken for tracks.

The anisotropic etching of fission tracks in different crystallographic orientations in zircons with low radiation damage was examined in more detail by progressively etching collimated ²⁵²Cf tracks in a large zircon crystal from the Mud Tank Carbonate in Central Australia. This zircon has a spontaneous track density of about $3 \times 10^6 \text{ cm}^{-2}$ and a correspondingly high level of radiation damage. One part of the zircon crystal was annealed at 850 °C for 1 h to simulate the absence of significant α -damage in the young zircons and to remove all preexisting fission tracks. The ²⁵²Cf tracks were then implanted on a polished (110) cleavage face in two orientations, parallel and perpendicular to the *c*-axis, both dipping at 30° to the surface. Figure 3 shows how one track etching parameter, the track density, varies with

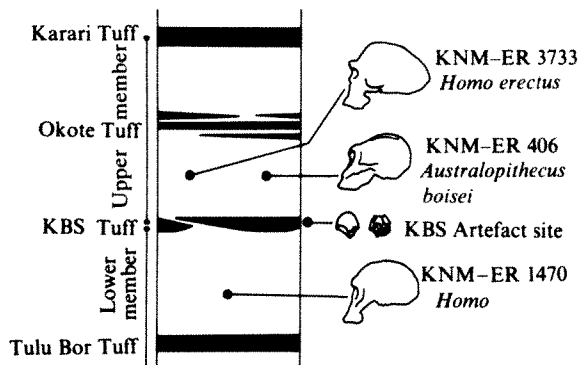


Fig. 1 Generalised stratigraphic column showing tuffs occurring in the Koobi Fora Formation to the east of Lake Turkana in northern Kenya. The approximate positions of some of the most important hominid and archaeological sites are also shown.

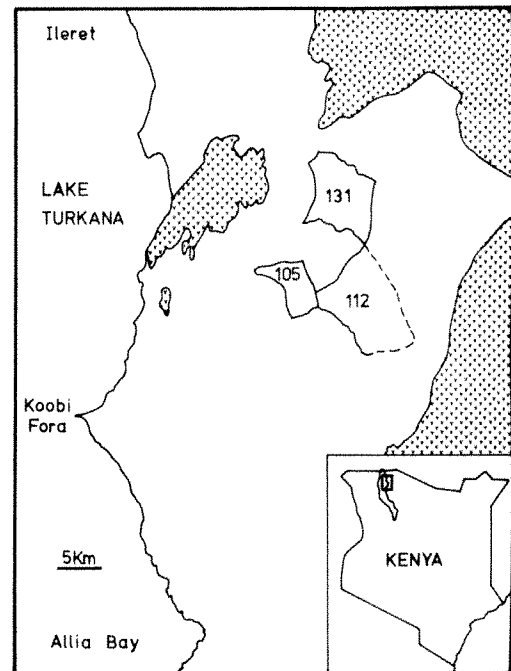


Fig. 2 Locality map of the East Turkana Basin showing the numbered fossil collection areas where the fission track dating samples were collected. Shaded areas indicate the older volcanic basement.

etching time for the two sets of ²⁵²Cf tracks compared with that for spontaneous tracks in an identical but unannealed sample of the zircon. The very different etching rates for the differently oriented tracks is obvious. The etched track shapes are also very different for the two orientations. Tracks perpendicular to the *c*-axis etch more readily and may appear quite satisfactory for counting before many of the tracks parallel to the *c*-axis are yet visible. With continued etching the *c*-parallel tracks become more like the acicular inclusions in appearance and great care is needed to discriminate between the two. These experiments enabled criteria to be set up for the reliable identification of etched tracks in all orientations and for their discrimination from spurious track-like features.

A small test mount was then made up of each of the zircons to be dated and this was irradiated with a high density of ²⁵²Cf tracks. This test mount was then etched alongside the main dating mount for each sample and was inspected periodically to judge the etching time required. Etching was continued for sufficient time (up to 100 h in KOH:NaOH eutectic at 210 °C) to ensure that the tracks in the slow-etching orientations were adequately revealed. The appearance of the etched ²⁵²Cf tracks in the auxiliary mount was then used as a guide to the expected appearance of fossil tracks in equivalent crystallographic orientations. This enabled a much more rigorous discrimination to be made between tracks and spurious features. A test of these discrimination procedures was made by 'dating' a sample of pumice zircon (FMA560) which had been annealed at 850 °C for 1 h to remove all spontaneous tracks.

Another significant departure from standard practice with the external detector method was to count every grain in the mount or in a predetermined area of the mount which was acceptable on the usual surface-orientation and etching criteria¹⁷. This avoided the selection of a small number of grains (typically only about five) for counting which is usually made with older (high track density) zircons. While this selection is inconsequential with high spontaneous track densities, it introduces a serious risk of bias where there are only a few tracks per grain or even no tracks in some cases. In addition all fission track counting of all samples was completed before any ages were calculated to guard against the possibility of earlier results influencing the identification of tracks in later determinations. After ~12 months the entire counting procedure, including the choice of

grains to be counted, was repeated for most of the samples as independently as possible from the first count. In this way duplicate, though not totally independent, results were obtained.

Neutron dosimetry procedures and the techniques used for apatite and sphene have been described in detail elsewhere¹⁸. Flux gradients during irradiation were monitored by using two dosimeter glasses. Approximately 1,200–1,600 tracks were counted over each dosimeter. Constants used in the age calculations were: $\lambda_F = 6.9 \times 10^{-17} \text{ yr}^{-1}$, $\lambda_D = 1.551 \times 10^{-10} \text{ yr}^{-1}$, $I = 7.253 \times 10^{-3}$, $^{235}\sigma = 5.802 \times 10^{-22} \text{ cm}^2$. Errors were calculated according to the method of Naeser *et al.*¹⁹ and McGee and Johnson²⁰ and are expressed as 1 standard deviation (σ) throughout.

KBS Tuff primary zircons

Analytical results are shown in Table 1 for primary igneous zircons from two individual and three composite pumice samples from the KBS Tuff. Results are also shown for two standard zircons of independently known age which were included in the same neutron irradiations as some of the KBS zircons. It can be seen in Table 1 that the fission track ages for these zircons from the Fish Canyon Tuff, Colorado (72N8) and the high level Mt Dromedary Intrusion in New South Wales (7822-9) are in excellent agreement with their K–Ar ages calculated using the new constants recommended by Steiger and Jäger²¹.

As expected no features which would definitely be counted as spontaneous tracks were found in the annealed sample of

FMA560 zircon. After annealing this zircon was subject to identical etching, irradiation and counting procedures as the other KBS Tuff zircons. Applying the same criteria as used for the other samples to discriminate tracks from spurious etch-pits, only four features were seen over 15 grains which might possibly have been confused with tracks and none of these were convincing. These would give a maximum contribution of only $0.11 \pm 0.05 \text{ Myr}$ to the age if all had been mis-identified as tracks. As even this background level is considered unlikely this experiment gives greater confidence that a reliable discrimination has been made for the dated KBS zircons.

For two of the KBS pumices (FMA559 and FMA560) sufficient zircon was obtained for more than one mount to be prepared and these were all etched and irradiated separately. Thus three independent age measurements were obtained for FMA559 and two for FMA560 giving eight measurements for the five pumices. Six of these were counted twice with very close agreement between the duplicate measurements in all but one case. For the second irradiation of FMA560 the duplicate results, which were counted over two quite separate sets of grains, are significantly different from each other at the 95% confidence level. The lower result, $1.54 \pm 0.10 \text{ Myr}$, seems anomalous. Excluding this result the zircon ages for the two individual pumice blocks from Area 131 are indistinguishable from each other and give a mean age and standard deviation of $1.85 \pm 0.06 \text{ Myr}$. This mean age is also indistinguishable from the ages of $1.85 \pm 0.06 \text{ Myr}$ and $1.90 \pm 0.08 \text{ Myr}$ obtained for the composite pumice samples from Areas 131 and 105 respec-

Table 1 Fission track ages of zircons from the KBS Tuff pumices and age standards

Rock no.	Area	No. of grains	R	$\rho_s \times 10^4$ (cm ⁻²)	$\rho_i \times 10^6$ (cm ⁻²)	$n \times 10^{15}$ (n cm ⁻²)	U (p.p.m.)	Calculated age (Myr $\pm 1\sigma$)	Average age (Myr $\pm 1\sigma$)
Individual pumices									
FMA559	131	14	0.666	8.78 (68)	3.64 (1411)	1.31	101	1.93 \pm 0.21	1.79 \pm 0.13
FMA559	131	19	0.944	9.22 (82)	4.30 (1910)	1.31	119	1.71 \pm 0.16	
FMA559	131	26	0.847	6.77 (115)	3.00 (2583)	1.37	80	1.86 \pm 0.15	1.86 \pm 0.15
FMA559	131	22	0.928	7.59 (193)	5.19 (6597)	2.05	92	1.83 \pm 0.12	1.83 \pm 0.12
FMA560	131	12	0.934	13.1 (156)	5.36 (3196)	1.31	149	1.95 \pm 0.14	1.91 \pm 0.10
FMA560	131	20	0.937	11.4 (143)	4.86 (3046)	1.31	135	1.87 \pm 0.14	
FMA560	131	15	0.892	8.58 (91)	5.73 (3039)	2.00	104	1.83 \pm 0.17	1.62 \pm 0.09
FMA560	131	24	0.973	8.62 (177)	6.84 (7019)	2.00	125	1.54 \pm 0.10	
Composite pumices									
FMA517	131	17	0.822	6.26 (137)	2.96 (3241)	1.42	76	1.83 \pm 0.14	1.83 \pm 0.10
FMA517	131	20	0.850	6.29 (139)	3.00 (3313)	1.42	77	1.82 \pm 0.13	
7722-107	131	15	0.943	13.50 (199)	8.40 (6199)	1.97	155	1.93 \pm 0.12	1.88 \pm 0.09
7722-107	131	15	0.936	12.5 (173)	8.24 (5688)	1.97	152	1.82 \pm 0.12	
7722-108	105	15	0.794	9.48 (121)	4.52 (2886)	1.44	114	1.84 \pm 0.15	1.90 \pm 0.08
7722-108	105	27	0.935	9.87 (277)	4.49 (6301)	1.44	114	1.93 \pm 0.10	
Annealed zircon									
FMA560	131	15	0.184	<0.424 (?4)	4.81 (2269)	1.98	89	<0.11 \pm 0.05	
Age standards									
72N8	—	6	0.912	623 (602)	28.3 (1367)	2.08	495	27.9 \pm 0.9	K–Ar age 27.8 \pm 0.4*
7822-9	—	5	0.807	2190 (1475)	23.5 (1587)	1.75	489	98.7 \pm 2.6	97.9 \pm 0.7†

R = correlation coefficient for spontaneous and induced track counts; ρ_s = spontaneous track density; ρ_i = induced track density; n = neutron dose; U = uranium concentration. Figures given in parentheses under each track density show number of tracks counted.

* Mean K–Ar age on biotite, hornblende, sanidine and plagioclase²⁶.

† Biotite K–Ar age²⁷.

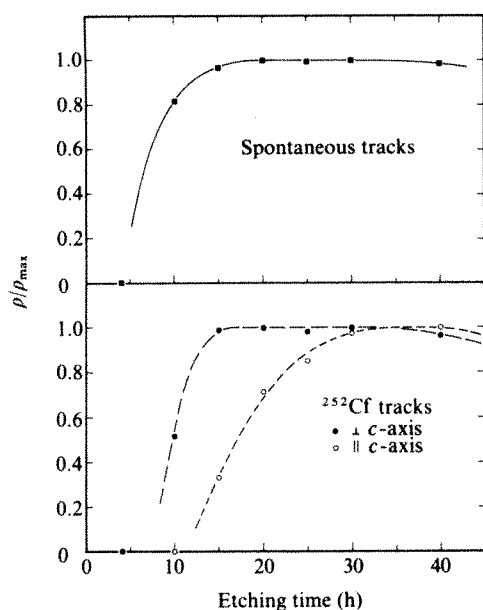


Fig. 3 Variation in observed track density (ρ) with etching time for spontaneous fission tracks and oriented ^{252}Cf tracks in an annealed sample of the same zircon. The track densities are expressed as a function of ρ_{max} , the maximum track density revealed.

tively. The close concordance of ages for the composite samples and those for individual pumice blocks strongly suggests that zircon-bearing pumice of only one age is present in the KBS Tuff.

A possible explanation for the anomalous FMA560 result of 1.54 ± 0.10 Myr may lie in the extreme range of uranium concentrations found in this sample. The 24 grains counted for this determination ranged from 54 to 516 p.p.m. uranium which is greater than for any other sample. This wide range in uranium concentrations is reflected in the high correlation coefficient observed in this case. It has been shown¹⁶ that the track etching rate in zircon varies with the total radiation damage so that

low-U grains etch more slowly than high-U grains for the same age. Thus it is possible that this determination included some low-U grains which were under-etched thereby depressing the combined age. The combined age of just the high-U grains alone (those with greater than the average of 125 p.p.m.) is 1.85 ± 0.17 Myr which supports this explanation.

The locality sampled in Area 105 can be traced directly to the type locality for the KBS Tuff but outcrop of this tuff is discontinuous between Areas 105 and 131. Nevertheless, the correlation is considered quite firm on the basis of stratigraphy⁷ and the compositions of pumice glasses²² and phenocrysts¹⁵. The dating results reported here also confirm the correlation between the two areas and, excluding the anomalous FMA560 value, give a mean age of 1.87 ± 0.04 Myr which is regarded as the best estimate of the fission track age of the KBS Tuff. Inclusion of the anomalous age would lower this mean to 1.83 ± 0.04 Myr which is not significantly different. The preferred mean fission track age of 1.87 ± 0.04 Myr is in close agreement with the mean K-Ar age of 1.89 ± 0.01 Myr reported by McDougall *et al.*²³ and that of 1.83 ± 0.06 Myr calculated from the data of Drake *et al.*²⁴ (all 1σ errors).

Detrital contamination

Some detrital contamination was present in all zircon concentrates dated, presumably from the small amounts of tuffaceous matrix which had worked deep into vesicles and fractures in the pumice. One of the major advantages of the external detector method of fission track dating, however, is that it permits the determination of ages on individual mineral grains, even at very small grain size ($<100 \mu\text{m}$). Thus it is possible to discriminate between the young primary igneous zircons and the old detrital zircons.

Two distinct groups of detrital zircons have been identified in the KBS Tuff and apart from these no other detrital grains have been found. The first consists of very rare zircon crystals which are euhedral and give single crystal ages between 24 and 27 Myr as shown in Table 2. Zircons of this age group were also found in mineral concentrates from pumices in the Tulu Bor Tuff, the Karari and its correlative, the Chari Tuff. Two apatite crystals of closely similar fission track age were found in pumice from the KBS Tuff in Area 105 (Table 2). These inherited zircons are thought to have been derived from a volcanic source probably to

Table 2 Fission track ages of detrital minerals occurring in pumices from the KBS Tuff

Rock no. 7722-	Area	Mineral	No. of grains	R	$\rho_s \times 10^6$ (cm^{-2})	$\rho_i \times 10^6$ (cm^{-2})	$n \times 10^{15}$ ($n \text{ cm}^{-2}$)	U (p.p.m.)	Age (Myr $\pm 1\sigma$)
'Volcanic' detritals									
108	105	Apatite	1	—	0.210 (18)	2.15 (92)	4.98	17	29.7 ± 8
108	105	Apatite	1	—	0.113 (4)	1.35 (24)	4.98	11	25.4 ± 14
108	105	Zircon	1	—	0.527 (34)	1.95 (63)	1.44	49	23.7 ± 5
108	105	Zircon	1	—	0.465 (24)	1.51 (39)	1.44	38	27.0 ± 7
108	105	Zircon	1	—	1.33 (180)	4.70 (318)	1.44	119	24.8 ± 2
'Basement' detritals									
108	105	Apatite	10	0.965	0.763 (504)	2.78 (918)	4.98	22	83 ± 3
108	105	Zircon	3	0.823	11.3 (399)	1.73 (918)	1.44	44	549 ± 32
108	105	Sphene	5	0.993	7.42 (813)	5.58 (611)	6.47	34	504 ± 10
109B	112	Zircon	6	0.887	19.5 (1078)	4.14 (407)	1.94	78	534 ± 17
109C	112	Zircon	3	0.999	29.6 (598)	3.07 (297)	1.92	58	456 ± 12
109C	112	Sphene	5	0.969	8.04 (830)	5.90 (609)	6.04	39	483 ± 11
107	131	Zircon	3	0.917	10.5 (711)	2.63 (237)	1.97	49	462 ± 22

Table 3 Fission track dating results for rocks from the Mozambique Belt basement in Kenya

Rock no.	Rock type	Mineral	No. of grains	<i>R</i>	$\rho_s \times 10^6$ (cm ⁻²)	$\rho_i \times 10^6$ (cm ⁻²)	$n \times 10^{15}$ (n cm ⁻²)	U (p.p.m.)	Age (Myr $\pm 1\sigma$)
100	Gneiss	Sphene	5	0.940	10.0 (2901)	6.77 (982)	5.52	49	479 \pm 12
101	Gneiss	Apatite	6	0.932	0.243 (365)	1.85 (1386)	8.75	8	70 \pm 2
102	Gneiss	Apatite	6	0.828	0.253 (334)	1.57 (1035)	8.69	7	85 \pm 3
103	Gneiss	Zircon	6	0.994	8.42 (1324)	1.21 (95)	1.26	35	514 \pm 40
103	Gneiss	Apatite	6	0.965	0.338 (446)	2.20 (1456)	8.63	10	80 \pm 2
104	Gneiss	Apatite	7	0.937	0.328 (533)	2.75 (2237)	8.56	13	62 \pm 2
136	Mag-Ap rock	Apatite	5	0.693	0.631 (1133)	3.27 (1470)	8.44	15	99 \pm 3
141	Granite gneiss	Sphene	5	0.950	20.5 (1621)	11.2 (1177)	5.07	88	542 \pm 12
141	Granite gneiss	Apatite	8	0.945	1.04 (484)	4.09 (952)	8.32	19	127 \pm 3

the east or north-east. No detrital ages younger than 24 Myr were found in zircon or apatite concentrates from the KBS Tuff. Miocene volcanics around the southeastern margin of the East Turkana Basin have given ⁴⁰Ar–³⁹Ar ages of 7.5 and 11.8 Myr (ref. 10) but a collection of these rocks proved to be almost totally lacking in zircon so that these are not a potential source of detrital contamination.

The other group of detrital zircons are much more abundant, often making up ~10–20% of these zircon concentrates. These are readily identified by their rounded, water-worn shapes, frequently darker colour and generally high spontaneous track densities. Two other minerals, sphene and apatite, with similar characteristics to these zircons are also found as detrital minerals in the pumice samples. Many of the detrital zircons are at least partly metamict and therefore unsuitable for fission track dating but satisfactory ages were obtained for some of the grains with low uranium concentrations. Table 2 shows fission track ages for all these detrital minerals, each age representing the combined counting results from several similar grains. Zircon and sphene ages from this group all fall in the range 500 \pm 50 Myr which is typical of radiometric ages for minerals from the crystalline rocks of the Mozambique Belt of East Africa²⁵. This is one of the 'Pan-African' mobile belts of late Precambrian–early Palaeozoic age and form the basement complex of the northern Kenya region.

Detrital apatites from the KBS Tuff in Area 105 (7722–108) give a combined age of 83 \pm 5 Myr which is not obviously related to either the 'Pan-African' detrital ages or the younger 'volcanic' detrital ages. However, apatites frequently give much younger fission track ages than sphenes or zircons from the same rocks due to the much lower thermal stability of tracks in apatite. Table 3 shows ages for apatite, sphene and zircon separated from basement gneisses of the Mozambique Belt from various parts of Kenya as shown in Fig. 4. It can be seen that sphene and zircon both show typical 'Pan-African' ages (500 \pm 50 Myr) but apatites give much younger ages ranging from 62 to 127 Myr with a weighted mean of 82 Myr. This pattern is almost identical to that found in the East Turkana detrital minerals. Thus it seems that the old detrital component found in the pumice samples has probably been derived from basement rocks of the Mozambique Belt.

Significance of the results

Two conflicting hypotheses have been offered to explain the different K–Ar and ⁴⁰Ar–³⁹Ar feldspar ages obtained for the KBS Tuff. Fitch *et al.*¹² consider the younger ages (~1.8 Myr) to be the result of a partial or total over-printing event. Curtis *et al.*¹¹, on the other hand, regard the older ages (~2.4 Myr) to be due to contamination of the feldspar separates by older material. It has also been pointed out that individual pumice blocks within

each of the reworked tuffs could conceivably be from more than one source and possibly of more than one age. Thus different ages might be obtained for a variety of analytical and geological reasons from samples collected at the one locality. K–Ar evidence of Curtis *et al.*¹¹ suggesting that tuffs mapped as the KBS in Areas 105 and 131 were of slightly different age, has now been eliminated with the discovery of a systematic error in the lower (1.6 Myr) ages²⁴.

The fission track evidence presented here has an important bearing on this discussion. There is very obvious agreement between the KBS Tuff zircon ages in Table 1 and the K–Ar ages of 1.8–1.9 Myr obtained by Curtis *et al.*¹¹, Drake *et al.*²⁴ and by McDougall *et al.*²³ which suggests that these ages date a real geological event. Also no fission track evidence was found for pumice of more than one age in the KBS Tuff from Area 131. Similarly there is no evidence that pumice from the KBS Tuff in Area 105, the type area for this tuff, has a different age from its

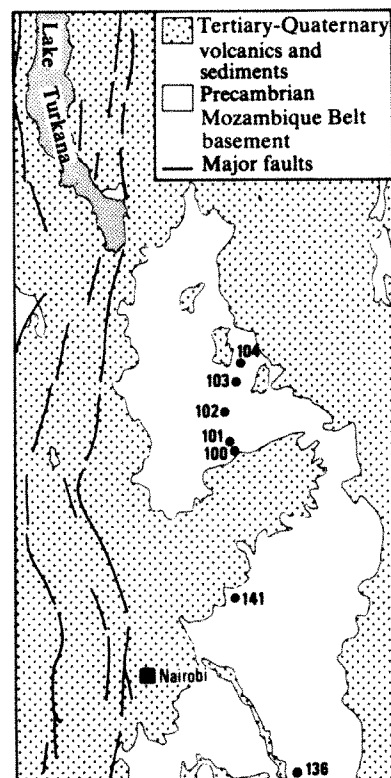


Fig. 4 Simplified geological map of part of Kenya showing localities of Precambrian basement rocks collected for fission track dating. Sample numbers have the prefix 7722-.

correlative in Area 131. All these observations argue against a purely geological explanation for the discrepancies in the published ages.

Table 2 clearly demonstrates the presence of detrital zircon, sphene and apatite grains in mineral concentrates separated from the pumice samples, even after very careful sample preparation. It therefore seems highly likely that feldspars separated for K-Ar dating could also contain traces of much older basement feldspar. This supports the contention that older K-Ar and ^{40}Ar - ^{39}Ar ages are the result of contamination. As discussed above the fission track ages of Hurford *et al.*¹⁴ are thought to be too old for purely analytical reasons, in particular the mis-identification of a finite number of acicular inclusions or dislocations as tracks and possibly a biased choice of grains for counting.

The presence of old detrital fission track ages also argues very strongly against the disturbance of any of the dating systems by postulated overprinting events. The pattern of ages found for detrital zircon, sphene and apatite closely matches that found in rocks of the Mozambique Belt basement which shows that the fission track ages have not been disturbed during erosion, deposition or any subsequent event. Considering the relative sensitivities of the different mineral dating systems to overprinting, it is inconceivable that total overprinting of glass and feldspar K-Ar ages at 1.8 Myr, as suggested by Fitch *et al.*¹², could have occurred without drastic effects on the detrital fission track ages, particularly those of the apatites. Thus the preservation of the old basement ages shows the hypothesis of post-depositional overprinting events to be unreasonable.

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Conclusions

Redetermination of the fission track age of primary igneous zircons from pumice contained in the KBS Tuff gives a mean age of 1.87 ± 0.04 Myr. This is regarded as representing the time of eruption of the pumice which in turn is taken to be a close approximation to the time of the deposition of this tuff in the East Turkana Basin. Individual pumice blocks from the KBS Tuff in Area 131 gave ages indistinguishable from each other and from pumice in Area 105.

The external detector method of fission track dating is particularly advantageous in detecting detrital contamination and discriminating against its effects on resulting ages. Two detrital components were identified, one showing essentially concordant zircon and apatite ages and probably from a volcanic source of dominantly early Miocene age. The other has a strongly discordant pattern of apatite ages compared with sphene and zircon which matches that observed for the basement rocks of the Mozambique Belt. The preservation of these detrital mineral ages fully justifies contamination as a plausible explanation for the older K-Ar ages and is strong evidence against the existence of previously postulated overprinting events.

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K-Ar age estimate for the KBS Tuff, East Turkana, Kenya

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Stone tools and numerous vertebrate fossils including hominids, have been found in close stratigraphic proximity to the KBS Tuff, whose age has been the subject of much debate. Concordant K-Ar ages, averaging 1.89 ± 0.01 Myr, are reported on anorthoclase phenocrysts from 13 pumice clasts collected from within the KBS Tuff or its correlatives. We believe that this age is the best estimate currently available for the time of formation of this important marker horizon within the East Turkana Basin.

PLIOCENE to Pleistocene sedimentary rocks which crop out adjacent to the eastern shores of Lake Turkana, northern Kenya, have yielded a wealth of vertebrate fossils, including hominids, and archaeological material¹⁻³. Rhyolitic tuffaceous beds within the sequence have facilitated mapping of the area¹⁻⁶, and these horizons contain samples amenable to

radiometric dating. The KBS Tuff⁴⁻⁶, within the Koobi Fora Formation, is one of these marker beds. As important hominid and artefact finds have been made within or adjacent to this stratigraphic level^{1-3,7,8}, it is of considerable importance to obtain precise and accurate ages for the KBS Tuff. Conventional K-Ar, ^{40}Ar / ^{39}Ar and fission track dating of pumice clasts within

this tuff have yielded a distressingly large range of ages⁹⁻¹⁴. In an attempt to resolve the controversy surrounding the age of the KBS Tuff we present here the results of a conventional K-Ar dating study of anorthoclase phenocrysts separated from pumice clasts found within the tuff.

KBS Tuff

Lake Turkana lies in a basin within the East African Rift System. During the late Pliocene and the Pleistocene the northeastern part of the Lake Turkana Basin was occupied by a large embayment in which sediments accumulated^{15,16} (Fig. 1). These sediments, about 325 m thick in all, are essentially flatlying, except locally, and represent a range of facies ranging from lacustrine through deltaic to fluvial.

The KBS Tuff is the topmost unit of the lower member of the Koobi Fora Formation^{4-6,15-18}, and is a medium to coarse grained vitric tuff of variable thickness, commonly about 1 m thick. Total thickness of overlying sediment does not exceed 125 m (ref. 18). In common with most tuffaceous horizons within the East Turkana sequence, the KBS Tuff is not a primary air fall tuff, but has been transported by, and deposited from, water¹⁸. Varying proportions of detrital material, derived from Miocene volcanic rocks and early Palaeozoic and Precambrian basement terrains marginal to the Lake Turkana Basin, are found within the tuffs.

Pumice clasts, up to 25 cm across, commonly water-rounded, occur locally within the KBS Tuff, and are regarded as products of the same volcanic eruptions that comprise the bulk of the tuff^{15,17,18}. The time between eruption of the primary volcanic material, including the pumice clasts and deposition within the East Turkana Basin, is considered to be very short—virtually instantaneous in terms of dating the deposits^{17,18}. Because the pumice clasts are less likely to be contaminated by detrital material compared with the enclosing tuff, most of the earlier age measurements were made on such clasts, and this is the procedure followed in this study. The clasts consist mainly of vesicular glass with variable proportions, normally less than 10% by volume, of anorthoclase phenocrysts up to 5 mm in size, and less common pyroxene. The glass is fresh to somewhat altered, and in some cases is partly replaced by carbonate. The phenocrysts are fresh and unaltered, the feldspar normally being limpid and euhedral. In the present study only the feldspar (anorthoclase) phenocrysts have been used for the dating.

Previous dating studies

Fitch and Miller⁹ dated a feldspar phenocryst concentrate, prepared from a pumice clast in the KBS Tuff, at 2.61 ± 0.26 Myr, subsequently¹² revising the age to 2.42 ± 0.01 Myr, based on results from four steps of a $^{40}\text{Ar}/^{39}\text{Ar}$ incremental heating experiment. They¹⁰ also reported $^{40}\text{Ar}/^{39}\text{Ar}$ total fusion ages on 10 feldspar concentrates from pumice clasts in the tuff, with results ranging from 0.52 ± 0.33 to 2.64 ± 0.29 Myr. In addition, conventional K-Ar ages of 8.43 ± 0.51 and 17.5 ± 0.9 Myr on feldspar from another clast (FM7054) were given¹⁰, but these old ages were thought to be caused by contamination from detrital material. Age spectra determined by the $^{40}\text{Ar}/^{39}\text{Ar}$ dating method were reported^{10,12} for feldspar concentrates from eight clasts from the KBS Tuff. Although the age spectra were stated to be complex, these authors interpreted apparent ages of 2.4–2.5 Myr for two samples (FMA274, FMA517) as crystallisation ages, and they suggested that overprinting could be recognised in most spectra at ~1.8–2.0 Myr, and in two cases at ~1.0 Myr. Support for this age of crystallisation of the pumices found within the KBS Tuff was given by Hurford *et al.*¹³, who reported fission track ages on zircon with a mean value of 2.44 ± 0.08 Myr.

Disregarding four conventional K-Ar ages on feldspar from pumice clasts in the KBS Tuff in the range 2.01–6.9 Myr, thought to be caused by detrital contamination, Curtis *et al.*¹¹ obtained concordant K-Ar ages on feldspar and glass from pumice clasts found in this horizon with mean values of 1.82 ± 0.04 Myr and 1.60 ± 0.05 Myr in two different areas, respec-

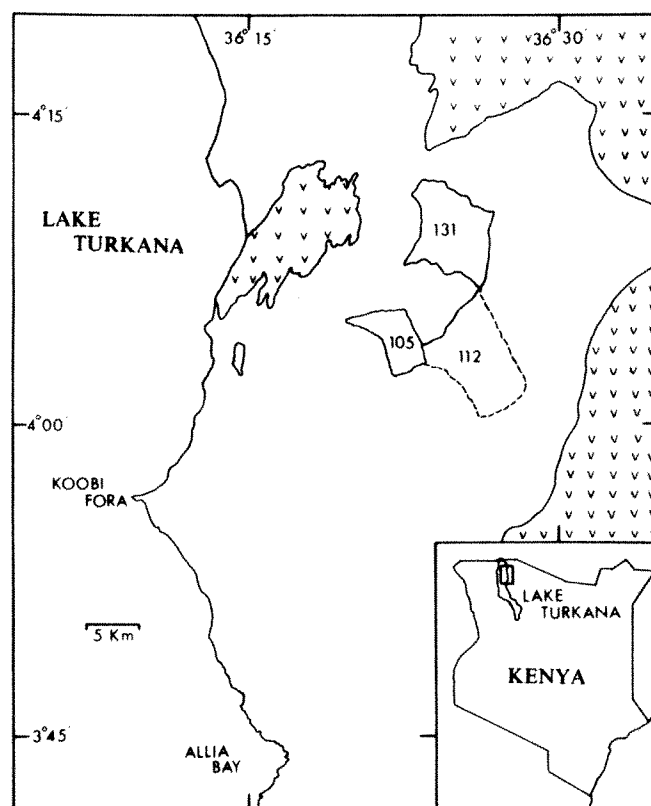


Fig. 1 Map of the Koobi Fora Region, adjacent to the northeastern shores of Lake Turkana, Kenya, showing location of the areas from which the samples for dating were obtained within the Pliocene-Pleistocene stratigraphic sequence (blank area). Volcanics forming the local basement are indicated by pattern. After Leakey and Leakey³.

tively. Subsequently, Drake *et al.*¹⁴ reported an error in potassium determinations on the samples previously dated by them¹¹ that yielded the 1.6 Myr ages. They listed¹⁴ new and revised K-Ar ages for 16 feldspar and six volcanic glasses from 13 pumice clasts in the KBS Tuff, ranging from 1.73 to 1.92 Myr, forming a reasonably concordant set with a mean of 1.8 ± 0.1 Myr.

Methods

Feldspar (anorthoclase) phenocrysts were separated from single pumice clasts found within the KBS Tuff and its correlatives in areas 105, 112 and 131, East Turkana (Fig. 1). In most cases the surface of the clasts was removed by sawing before crushing. Following crushing, anorthoclase was concentrated by a panning procedure, and normally the +1 mm fraction was retained and further crushed to $-360 + 170 \mu\text{m}$ or $-360 + 100 \mu\text{m}$. Tetra-bromoethane, adjusted to appropriate density, was used to purify the anorthoclase. The final step involved treatment of the concentrate in dilute HF (7%) ultrasonically for 5 min. Grain counting showed that the separates had a grain purity of better than 99.5%, and that glass remaining on crystal faces or as small inclusions rarely exceeded 1%.

Potassium was determined by flame photometry using Li internal standard and Na buffer on an IL 443 machine, following procedures similar to those previously described¹⁹. Argon extractions were made on aliquants of 0.9–2.9 g in a high vacuum line by radiofrequency heating. Temperatures of ~1,500°C were maintained for 30 min to ensure complete outgassing of argon from the melt. Previously it had been found²⁰ that temperatures substantially greater than that needed for fusion were required to remove all argon from K-feldspars. A tracer of ^{38}Ar , prepared from a gas pipette system, was added during the fusion. Subsequent to purification of the argon, its isotopic composition was measured in a AEI MS10 or a Micromass 12 mass spectrometer, both

operated in the static mode. Peak hopping is used on both machines and results are taken digitally on-line with a Hewlett-Packard 21MX computer, in which all data processing is done to produce an age within minutes of completion of the isotopic analysis.

Errors are quoted at the level of one standard deviation as previously described^{21,22}. The average age given in those cases where the sample was measured in duplicate has a minimum assigned error of 1%, because the uncertainties in the measurement of potassium and calibration of the ³⁸Ar tracer are of this order.

All ages are calculated using the revised ⁴⁰K decay constants and isotopic abundances, recently recommended²³. These constants yield ages 2.67% greater than those reported by most previous workers, but Drake *et al.*¹⁴ have also used the new constants.

Results and discussion

Petrographically the K-feldspar phenocrysts in the pumice clasts are homogeneous with no evidence of unmixing. Extremely fine polysynthetic twinning is observed in some crystals. X-ray diffraction measurements, using the three peak method of Wright²⁴, carried out by W. D. Birch (personal communication), indicate that the feldspar is highly disordered and structurally close to the sanidine-high albite series, exhibiting no evidence for significant unmixing or strain resulting from exsolution. The feldspar has a composition in the range Or₃₅–Or₃₉ (weight %) from X-ray diffraction analysis, comparing well with the composition of Or₃₅–Or₃₇ derived from the potassium analyses (Table 1). Following the nomenclature of Wright and Stewart²⁵ these feldspars are classified as anorthoclase.

Results of the K–Ar dating are given in Table 1. Duplicate potassium measurements agree to better than 1%, and the range of values is limited to less than 4% relative. In most cases sufficient anorthoclase was available to enable argon to be measured in duplicate; generally these agree to better than 2%.

With few exceptions, which will be discussed first, the K–Ar ages on 17 anorthoclase concentrates from 14 different pumice clasts collected from the KBS Tuff or its correlatives are remarkably concordant at ~1.9 Myr (Table 1).

Anorthoclase from sample KF43 yields anomalously old apparent ages of 4.11 and 7.46 Myr (Table 1). We attribute these poorly reproducible ages to the presence of variable but small amounts of old detrital K-feldspar in the aliquants used in the argon extractions. Careful petrographic examination of the mineral concentrate, however, did not lead to positive identification of detrital K-feldspar. Nevertheless, there is no doubt that old detrital material was being brought into the East Turkana Basin during deposition of the sediments. For example, we have obtained a K–Ar age of 456 ± 5 Myr (K = 10.73%; radiogenic ⁴⁰Ar = 9.68 × 10⁻⁹ mol g⁻¹) on a large (30 mm) crystal of microcline (sample no. 79–457) found weathering out of sediments of the Koobi Fora Formation. Such an age is typical of the Mozambique Belt of East Africa^{26,27}. Contamination of the anorthoclase concentrate from KF43 by less than 0.5% of old K-feldspar of this kind would explain the aberrant results.

Two anorthoclase separates were obtained from pumice clast 7722–109C, one from the outer part of the clast (79–19), yielding an age of 2.00 ± 0.02 Myr, and the other (79–276) from the core of the clast, giving an age of 1.89 ± 0.02 Myr. These results clearly demonstrate that the outer layers of the pumice cobbles may contain detrital feldspar.

For two pumice clasts (KF38, KF47) two size fractions of feldspar were analysed, and in each case the finer fraction yields slightly older apparent ages. For KF47 the difference in measured age for the two size fractions is barely significant, but for KF38 the ages differ by 4.5%, and this difference is regarded as highly significant. This discrepancy may be because of incomplete extraction of the argon from the coarser fraction or because of minor contamination by detrital feldspar in the finer fraction. The latter explanation is preferred but cannot be proved.

Table 1 K–Ar ages on anorthoclase phenocrysts separated from pumice clasts found within the KBS Tuff or its correlatives, East Turkana, Kenya

Lab. no.	Field no.	Size fraction (μm)	K (wt %)	Wt used in Ar extraction (g)	Radiogenic ^{40}Ar (10^{-11} mol g $^{-1}$)	100 Rad. ^{40}Ar	Calculated age (Myr) ± 1 s.d.	Average age (Myr) ± 1 s.d.
						Total ^{40}Ar		
Area 105								
78-1038	KF38	210-350	5.179, 5.147	2.664	1.700	48.6	1.90 \pm 0.02	1.89 \pm 0.02
				2.869	1.690	85.7	1.89 \pm 0.02	
				2.515	1.778	77.5	1.99 \pm 0.03	
78-1038	KF38	100-210	5.174, 5.128	2.606	1.762	40.9	1.97 \pm 0.03	1.98 \pm 0.02
Area 112								
79-17	7722-109A	125-350	5.108, 5.112	1.013	1.651	35.6	1.86 \pm 0.02	1.87 \pm 0.02
				1.020	1.662	37.5	1.87 \pm 0.02	
79-18	7722-109B	100-350	4.975, 4.949	0.904	1.622	41.1	1.88 \pm 0.02	1.88 \pm 0.02
79-19	7722-109C	100-350	5.163, 5.163	0.912	1.795	64.6	2.00 \pm 0.02	2.00 \pm 0.02
79-276	7722-109C	100-350	5.107, 5.112	1.931	1.682	84.4	1.90 \pm 0.03	1.89 \pm 0.02
				2.106	1.675	84.9	1.89 \pm 0.02	
				2.114	1.679	83.0	1.89 \pm 0.02	
79-277	7722-109D	180-350	5.100, 5.115	2.080	1.674	84.6	1.89 \pm 0.02	1.89 \pm 0.02
78-1039	KF39	210-420	5.042, 5.042	2.028	1.636	68.3	1.87 \pm 0.02	1.87 \pm 0.02
78-1040	KF40	180-350	5.100, 5.138	1.806	1.670	85.2	1.88 \pm 0.03	1.89 \pm 0.02
				2.212	1.684	84.5	1.90 \pm 0.03	
78-1041	KF41	180-350	5.097, 5.093	1.979	1.655	86.7	1.87 \pm 0.02	1.88 \pm 0.02
				1.696	1.666	83.3	1.88 \pm 0.02	
78-1043	KF43	210-420	5.061, 5.042	1.859	6.556	87.9	7.46 \pm 0.08	—
				0.894	3.723	90.6	4.11 \pm 0.04	
Area 131								
78-1044	KF44	100-500	5.136, 5.152	1.660	1.703	63.5	1.91 \pm 0.02	1.90 \pm 0.02
				2.239	1.687	82.2	1.89 \pm 0.02	
78-1047	KF47	210-350	5.138, 5.112	2.399	1.673	78.5	1.88 \pm 0.02	1.88 \pm 0.02
				2.581	1.663	46.4	1.87 \pm 0.02	
78-1047	KF47	100-210	5.117, 5.155	2.528	1.693	53.8	1.90 \pm 0.03	1.91 \pm 0.02
				2.540	1.719	63.6	1.93 \pm 0.03	
78-1048	KF48	210-350	5.127, 5.127	2.576	1.681	72.9	1.89 \pm 0.02	1.92 \pm 0.02
				2.403	1.731	75.5	1.94 \pm 0.02	
79-14	7722-107I	100-350	5.146, 5.171	1.013	1.671	55.5	1.87 \pm 0.02	1.88 \pm 0.02
				1.022	1.700	55.2	1.90 \pm 0.02	
79-15	7722-107II	100-350	5.129, 5.173	1.017	1.665	62.5	1.86 \pm 0.03	1.88 \pm 0.02
				2.571	1.695	77.0	1.90 \pm 0.03	

$$\lambda_e + \lambda_c = 0.581 \times 10^{-10} \text{ yr}^{-1} \quad \lambda_\beta = 4.962 \times 10^{-10} \text{ yr}^{-1} \quad {}^{40}\text{K}/\text{K} = 1.167 \times 10^{-4} \text{ mol mol}^{-1}$$

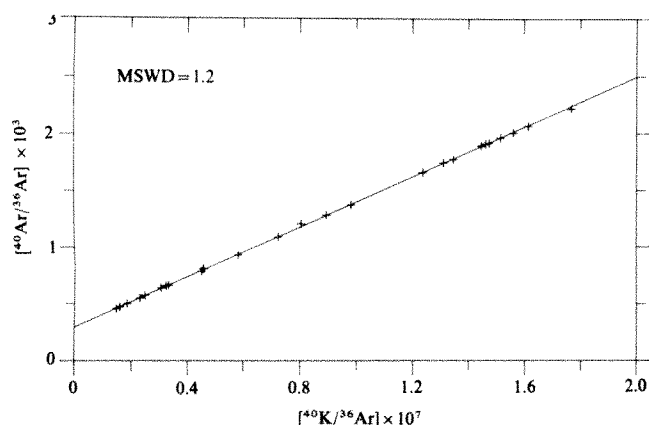


Fig. 2 K-Ar isochron diagram showing data from anorthoclase phenocrysts from pumice clasts found within the KBS Tuff. $R_1 = 293.8 \pm 2.4$; age = 1.89 ± 0.01 Myr.

Thus the K-Ar results from KF43, 79-19 and the finer fraction of KF38 are excluded from further consideration.

The average K-Ar ages for the other 14 anorthoclase separates from 13 different pumice clasts collected from the KBS Tuff, lie within the narrow range of 1.88–1.92 Myr, with a mean age of 1.89 ± 0.01 Myr (Table 1). Applying the isochron approach²¹ to these same data, yields a least squares fitted²⁸ line whose slope is equivalent to an age of 1.89 ± 0.01 Myr (Fig. 2), where the uncertainty quoted is at the 95% confidence level. Note that the line fits to within experimental error, as the value of the mean square of weighted deviates (MSWD) does not differ significantly from unity, and that the age is identical to that derived simply by averaging the K-Ar ages calculated for individual anorthoclase separates. In the isochron analysis no assumption is made concerning the $^{40}\text{Ar}/^{36}\text{Ar}$ ratio of the non-radiogenic component. Thus the fact that the value found (293.8 ± 2.4) is indistinguishable from that of atmospheric argon (295.5) provides independent verification that in the present case the non-radiogenic argon has atmospheric composition, as normally assumed in the calculation of K-Ar ages.

The concordancy of the K-Ar results argues strongly against the presence of contamination by old detrital K-feldspar, as it would be most unlikely that the proportion of contamination would be so uniform. Because all these samples consist of clear, fresh, high-temperature, volcanic anorthoclase, which normally has excellent retention properties for radiogenic argon, the measured age is interpreted as that of the eruptive episode that produced the pumice clasts and the enclosing tuff. The KBS Tuff has never been buried deeper than about 125 m, so that temperatures since deposition are not likely to have been raised much above ambient. We conclude, therefore, that loss of radiogenic argon from the anorthoclase phenocrysts by reheating subsequent to deposition of the pumice clasts, as proposed by Fitch and Miller¹⁰, is geologically unreasonable.

On the basis of chemical data on pumices, Cerling *et al.*²⁹ have argued that the tuff in area 112 (referred to as area 105-East in their paper) is not the same horizon as the KBS Tuff in areas 105 and 131. Our results on anorthoclase phenocrysts from the pumices in the three areas show no significant differences in potassium content or in measured K-Ar age. Thus our data strongly support the view of I.C. Findlater (personal communication) that the tuff in area 112 is stratigraphically equivalent to the type KBS Tuff in area 105.

Comparison with other results

The mean K-Ar age of 1.89 ± 0.01 Myr, obtained in the present study for anorthoclase separates from 13 pumice clasts in the KBS Tuff and its correlatives, is statistically indistinguishable from the mean K-Ar age of 1.83 ± 0.06 Myr calculated from the data of Drake *et al.*¹⁴, based on 22 measurements of feldspar and glass from 13 pumice clasts in the same horizon. Nevertheless,

the spread in apparent age for the samples analysed by Drake *et al.* is significantly greater than that found in the present study. Their reported ages for glass range from 1.73 ± 0.04 to 1.90 ± 0.03 Myr (six glass fractions from four samples), and for feldspar the ages range from 1.73 ± 0.03 to 1.92 ± 0.02 Myr (16 feldspar concentrates from 13 samples), a spread of some 10%, and much greater than the quoted errors of 1–2% for individual ages. It is significant that the oldest ages reported by Drake *et al.* are in excellent agreement with those found in the present study, and that the spread is towards younger ages.

Direct comparison between the results of Drake *et al.*¹⁴ and those determined in this laboratory is possible because measurements have been made in both laboratories on three pumice clasts which were collected jointly from the KBS Tuff by T. E. Cerling and A. J. W. G., and split into two. Separates for dating were prepared independently in the two laboratories, and the results are summarised in Table 2. The differences in measured potassium content for the feldspars are small and may simply reflect slight differences in the purity of the separates. However, the differences in reported radiogenic argon content range from 5.7 to 11%, with our results higher.

Table 2 Comparison of K-Ar ages obtained on separates from the same pumice clasts in the Berkeley and Canberra Laboratories

Berkeley no.	Canberra no.	Sample	K (wt%)	Radiogenic ^{40}Ar (10^{-11} mol g $^{-1}$)	Calculated age (Myr \pm 1 s.d.)
KBS 77-109A		Feldspar	4.96	1.54	1.79 ± 0.02
	7722-109A	Feldspar	5.11	1.65	1.87 ± 0.02
		Glass	4.04	1.33	1.90 ± 0.03
KBS 77-109B		Feldspar	4.87	1.46	1.73 ± 0.03
	7722-109B	Feldspar	4.96	1.62	1.88 ± 0.02
KBS 77-109D		Feldspar	5.12	1.59	1.79 ± 0.02
	7722-109D	Feldspar	5.11	1.68	1.89 ± 0.02

Two points may be made. First, the Canberra results have a mean measured age of 1.88 ± 0.02 Myr for the three feldspars, whereas those measured in Berkeley by Drake *et al.*¹⁴ yield a mean of 1.77 ± 0.03 Myr, a difference of some 6%. Second, the age of volcanic glass from sample KBS 77-109B of 1.90 ± 0.03 Myr is concordant with the Canberra feldspar ages, but 9.8% greater than the age measured in Berkeley on feldspar from the same sample. Because glass normally is much more susceptible to loss of radiogenic argon than high temperature feldspar, it is indeed curious that the feldspar, as measured in Berkeley, yields a lower apparent age than the coexisting glass. A possible explanation for these results is that extraction of radiogenic argon from the feldspars was incomplete in the Berkeley experiments, leading to low apparent ages. We have mentioned previously the difficulty of extracting the argon quantitatively from feldspars of this kind, even when the samples have been melted in the vacuum system. Further intercomparisons are necessary to clarify this problem, but we wish to stress that overall the K-Ar age results from the two laboratories are in good agreement, and provide convincing evidence that the KBS Tuff has an age in the range 1.8–1.9 Myr.

It now seems clear that the earlier K-Ar and $^{40}\text{Ar}/^{39}\text{Ar}$ total fusion results on feldspar from pumice clasts of the KBS Tuff reported by Fitch and Miller^{9,10}, and Fitch *et al.*¹², must be regarded as imprecise, as is evident from the large scatter of their data. This imprecision arises partly from the high atmospheric argon contamination found in most of their argon analyses, generally in the range 80–95% of the total ^{40}Ar . The meaning of the complex $^{40}\text{Ar}/^{39}\text{Ar}$ age spectra found^{9,10,12} for anorthoclase remains unclear. Three of the samples yield apparent ages for part of the age spectra between 2.4 and 2.5 Myr, possibly reflecting some detrital contamination. Fitch and Miller¹⁰ interpret apparent ages of around 1 Myr and between 1.8 and 2 Myr in some of the age spectra as indicating overprinting with loss of radiogenic argon at these times. On the basis of our data we believe the apparent ages of 1.8–2 Myr are recording the time of crystallisation and cooling of the feldspars, rather than

overprinting. It has been noted that the postulated overprinting events seem unreasonable on geological grounds. Indeed the presence of fresh glass in some of the pumice clasts, together with the relatively good agreement between the K-Ar ages on the glass and coexisting feldspar, as reported by Drake *et al.*¹⁴, provides powerful evidence against such overprinting.

Finally, the recently re-determined fission track ages, averaging 1.87 ± 0.04 Myr, on zircon separated from pumice clasts in the KBS Tuff, reported by Gleadow²⁷, are in excellent agreement with our K-Ar data, and resolve an outstanding problem.

Conclusions

The mean K-Ar age of 1.89 ± 0.01 Myr found in the present study for anorthoclase separates from 13 pumice clasts of the KBS Tuff is interpreted as the age of crystallisation and cooling of the pumices, which were deposited shortly thereafter in the East Turkana Basin. We believe that these data provide the best estimate presently available for the age of this stratigraphically important horizon within the East Turkana sedimentary

sequence. This view is reinforced by the satisfactory agreement between our results and those obtained by Drake *et al.*¹⁴ in the Berkeley laboratory on anorthoclase and glass, and the fission track ages on zircon given by Gleadow²⁷. The combined results seem to resolve the long-standing controversy surrounding the age of the KBS Tuff and indicate that this horizon was deposited in the late Pliocene as the Plio-Pleistocene boundary has an estimated age of ~ 1.6 – 1.7 Myr (ref. 30). Acceptance of such an age for the KBS Tuff also resolves the correlation problems, recently summarised by Behrensmeyer³¹ and Brown *et al.*³², between the sequences in East Turkana and the Omo area of southern Ethiopia. The present results also suggest that the KBS Tuff was deposited during the time interval represented by the Olduvai normal polarity event in the Matuyama reversed epoch of the geomagnetic polarity time scale^{33–36}.

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Chicken lens crystallin DNA sequences show at least two δ -crystallin genes

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Restriction analysis of chicken DNA using probes derived from a cloned cDNA and analysis of cloned genomic DNA fragments containing δ -crystallin gene sequences have indicated the presence of at least two non-allelic genes for δ -crystallin, the first and principal crystallin synthesised in the embryonic chicken lens. Electron microscopic analyses of three cloned genomic fragments revealed that the δ -crystallin mRNA gene sequences are interrupted at least 14 times in one of the δ -crystallin genes.

CRYSTALLINS are a group of structural proteins which constitute the bulk of the protein in the vertebrate lens¹. Since they are tissue specific, the crystallins are advantageous markers for cellular differentiation^{2–5}. δ -Crystallin, found only in birds and reptiles^{6–8}, is a particularly useful index of lens cell differentiation. It is the first crystallin to appear after lens induction in the chicken embryo^{9–11}. Further differentiation of lens fibre cells is correlated with a rapid accumulation of δ -crystallin mRNA^{12–14}. δ -Crystallin represents 70 to 80% of the protein synthesised by the embryonic lens fibre cells which, by 15 days of

development, contain on average $1\text{--}2 \times 10^5$ molecules of δ -crystallin mRNA per cell¹⁵. The embryonic chicken lens fibre cells synthesise much smaller amounts of α - and β -crystallin¹⁶. After hatching, δ -crystallin synthesis decreases and β -crystallin synthesis predominates¹⁶. δ -Crystallin is composed of at least two similar polypeptides^{17,18}. Although δ -crystallin mRNA has not been fractionated into more than one species^{13,19}, it is possible that the δ -crystallin polypeptides may be encoded by similar but non-identical mRNAs¹⁵.

An understanding of the regulation of δ -crystallin synthesis

during development will ultimately require knowledge of the structure of the δ -crystallin gene(s). Annealing experiments with cDNA have shown that the δ -crystallin gene sequences are situated in the non-reiterated region of the genome²⁰, but the actual number and organisation of δ -crystallin gene(s) have not been established. We have recently cloned δ -crystallin cDNA in a bacterial plasmid²¹, which provides an opportunity for analysis of the natural δ -crystallin gene(s). In this article we have used this recombinant plasmid to investigate the organisation of the δ -crystallin sequences in chicken DNA and in genomic fragments cloned from it.

Identification and cloning of δ -crystallin gene sequences

High molecular weight DNA was extracted from erythrocytes of White Leghorn chickens (gs⁻, Spafas Incorporated). The DNA was limit-digested with *Eco*RI and chromatographed on an RPC-5 column followed by agarose gel electrophoresis²². The fragments containing δ -crystallin sequences were identified by Southern blotting²³ and hybridisation to δ -crystallin ³²P-cDNA (ref. 21), synthesised from sucrose density gradient purified mRNA¹⁹. Four principal bands of DNA hybridised to the ³²P-cDNA (data not shown). Four major *Eco*RI fragments containing δ -crystallin sequences were also found with DNA isolated from pooled embryos or from adult liver or cultured embryonic fibroblasts derived from individual chickens. In these experiments nick-translated p δ Cr-2 DNA was used as a probe. p δ Cr-2 is a recombinant plasmid containing approximately 1.3 kilobases of δ -crystallin cDNA cloned in the bacterial plasmid pBR322 (ref. 21). δ -Crystallin mRNA is approximately 2 kilobases long^{19,21}. p δ Cr-2 DNA was used as a probe for all subsequent experiments.

The fragments were purified from the appropriate RPC-5 column fractions of the erythrocyte DNA by preparative agarose gel electrophoresis²⁴ and identified by analytical agarose gel electrophoresis, Southern blotting and hybridisation to ³²P-cDNA as above. We used the *in vitro* packaging method of Blattner *et al.*²⁵ to clone the two largest fragments in bacteriophage λ Charon 4A and the third largest fragment in bacteriophage λ gtWES (ref. 26). Charon 4A was selected because the lengths of the two biggest fragments were initially thought to be larger than 10 kilobases. Re-electrophoresis of the purified fragments containing δ -crystallin sequences indicated that their lengths were approximately 12, 7.5, 4 and 3 kilobases. The recombinant clones were identified by the *in situ* screening procedure²⁷. We refer to the cloned 7.5-, 12- and 4-kilobase fragments as g δ Cr-1, g δ Cr-2 and g δ Cr-3 respectively (for genomic δ -crystallin clones 1, 2 and 3).

To determine the relative orientation of the 7.5-, 12- and 4-kilobase fragments in the genome, we took advantage of an *Eco*RI site within the cloned cDNA insert in p δ Cr-2 (ref. 21). Digestion of p δ Cr-2 with *Eco*RI generates one fragment containing approximately 550 base pairs from the 3' half of the cloned δ -crystallin cDNA and another fragment containing approximately 700 base pairs from the 5' half of the cloned cDNA. The extreme 5' sequences of the δ -crystallin mRNA are not represented in p δ Cr-2. The 5' probe derived from p δ Cr-2 hybridised strongly to the cloned 7.5-kilobase fragment, extremely weakly to the 12-kilobase fragment and not at all to the 4-kilobase fragment. The 3' probe hybridised exclusively to the 12- and 4-kilobase cloned fragments. We conclude from this experiment that the 7.5-kilobase fragment contains δ -crystallin sequences located 5' to those in the 12- or 4-kilobase fragments with respect to δ -crystallin mRNA.

Visualisation of δ -crystallin sequences in the genomic clones

Electron microscopy was used to investigate the organisation of the sequences complementary to δ -crystallin mRNA within the cloned fragments. Heteroduplexes were formed between the DNAs of g δ Cr-1 and g δ Cr-2, and g δ Cr-1 and g δ Cr-3;

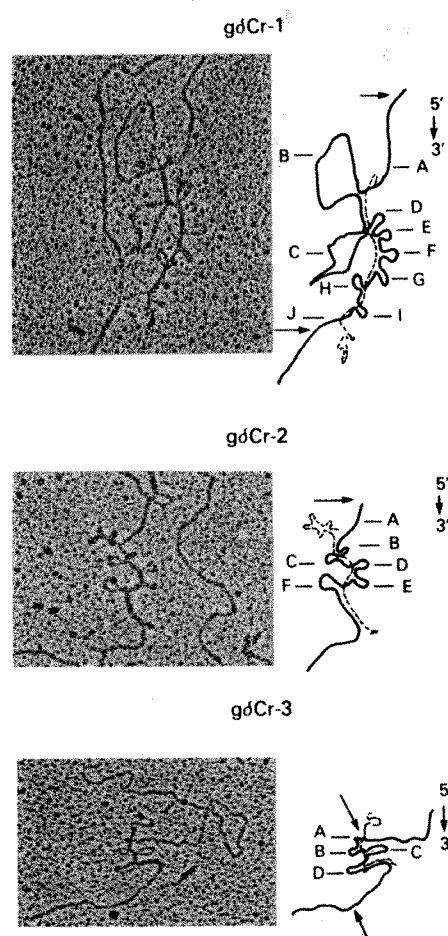


Fig. 1 Electron micrographs of the hybrids formed between δ -crystallin mRNA and the 7.5-kilobase fragment in g δ Cr-1, the 12-kilobase fragment in g δ Cr-2 and the 4-kilobase fragment in g δ Cr-3, respectively. Heteroduplexes were formed between g δ Cr-1 and g δ Cr-2 or g δ Cr-1 and g δ Cr-3 DNA ($10 \mu\text{g ml}^{-1}$ each) in 70% formamide, 0.1M Tris-HCl, pH 7.5, 0.15M NaCl, 0.01M EDTA and $25 \mu\text{g ml}^{-1}$ of δ -crystallin mRNA. The cloned DNAs were first kept at 85°C for 10 min and then the mRNA was added to the reaction mixture. The hybridisation was continued at 43°C for 2 h. Reaction mixtures were diluted 1:20 in the hybridisation buffer, prepared for electron microscopy²¹ and examined in a Philips 300 electron microscope. An illustrative tracing is presented for each electron micrograph. The intervening sequences (IVS) in each molecule are indicated by letters. IVS B in g δ Cr-2 was observed in only one-third of the molecules. This may be due to the short hybridised region between A and B. Alternatively, B may be a spreading artefact. It is even possible that its occasional presence is due to the existence of two different δ -crystallin mRNAs. Digestion of g δ Cr-1 by *Eco*RI released two cloned fragments of about 8.0 kilobases indicating end-to-end ligation of two fragments. This explains the viability of the 7.5-kilobase fragment in λ Charon 4A, which requires at least 10 kilobases of inserted DNA. Only one of these fragments (7.5 kilobases long) contained δ -crystallin sequences. Similarly, two cloned fragments of 5.0 kilobases and 4.0 kilobases were released when g δ Cr-3 was digested by *Eco*RI. Out of these two fragments only the 4-kilobase fragment contained δ -crystallin sequences. Arrows indicate the *Eco*RI sites: —: single-stranded DNA; --- mRNA.

δ -crystallin mRNA was then hybridised to the single-stranded region of each of the heteroduplexes. The upper panel of Fig. 1 shows an electron micrograph of a hybrid between δ -crystallin mRNA and the complementary sequences in g δ Cr-1 DNA. The RNA-DNA hybrids were interrupted by eight DNA loops, indicating discontinuity of the sequences complementary to δ -crystallin mRNA. These intervening sequences (IVS) are denoted by letters A-J; the hybridised regions are denoted by numbers 1-9 (see Fig. 2). The cumulative length of the hybri-

disised regions, which we call mRNA gene sequences (mRGS), was 1.4 ± 0.4 kilobases (mean \pm s.d.); the length of the intervening sequences was 6.2 ± 0.9 kilobases. The long RNA tail is the 3' part of δ -crystallin mRNA, since more than 60% of the mRNA sequences hybridised to g δ Cr-1, and g δ Cr-1 did not hybridise to the 3' half of the cloned δ -crystallin cDNA. The non-hybridised stretch of DNA-labelled A may be an intervening sequence because a short tail of RNA was occasionally observed to the left of mRGS 1. This suggests that a δ -crystallin mRNA gene sequence is missing from the 5' end of the 7.5-kilobase fragment. Assuming that the sequences at the extreme 5' end of δ -crystallin mRNA are not present in g δ Cr-1, IVS A would be the largest intervening sequence (at least 1.5 kilobases) in g δ Cr-1. It is interesting that the intervening sequence separating the 45-base pair leader sequence of ovalbumin mRNA in the chicken genome is 1.6 kilobases long²⁸.

The electron micrograph in Fig. 1 (middle) shows a hybrid between δ -crystallin mRNA and the single-stranded DNA in g δ Cr-2. The prominent RNA tail seen in this hybrid is the 5' end of δ -crystallin mRNA, since only the 3' half of the cloned δ -crystallin cDNA hybridises well to this fragment. The small knob-like structure present at the 3' end of the hybrid probably represents the poly(A) tail of the mRNA. Six intervening sequences (A-F) were observed in this hybrid. The cumulative length of the intervening sequences in g δ Cr-2 was 1.9 ± 0.3 kilobases and that of the mRNA gene sequences was 0.8 ± 0.2 kilobases.

The electron micrograph in Fig. 1 (lower) shows a hybrid between δ -crystallin mRNA and the single-stranded DNA in g δ Cr-3. The δ -crystallin sequences within this cloned 4-kilobase fragment hybridised only to the 3' half of the cloned cDNA probe. The long RNA tail is thus the 5' side of the δ -crystallin mRNA. Four intervening sequences (A-D) interrupted the δ -crystallin mRNA gene sequences in this cloned fragment. By analogy with g δ Cr-2, there may be a fifth intervening sequence to the left of the mRNA gene sequence 1 (see Fig. 2) in g δ Cr-3. This cannot, however, be established by electron microscopy. The cumulative length of the intervening sequences of the cloned 4-kilobase fragment is about 1.7 ± 0.3 kilobases, and that of the mRNA gene sequences is about 0.6 ± 0.1 kilobases—values in close agreement with the corresponding values for the 12-kilobase fragment. The 12- and 4-kilobase fragments thus appear to contain very similar but not identical 3' halves of separate δ -crystallin genes (see below).

Diagrammatic representations of the δ -crystallin gene sequences in the three cloned fragments are shown in Fig. 2. δ -Crystallin DNA sequences in g δ Cr-1 must be associated with those in g δ Cr-2 or g δ Cr-3 to form one of the natural δ -crystallin genes. Either combination contains a sufficient number of δ -crystallin mRNA gene sequences to account for a δ -crystallin gene, except probably the 5' leader sequence. The cumulative length of the mRNA gene sequences in the 7.5 (g δ Cr-1) and the 12 (g δ Cr-2) kilobase fragments is 2.2 ± 0.6 kilobases, and the cumulative length of these sequences in the 7.5 and the 4 (g δ Cr-3) kilobase fragments is 2.0 ± 0.5 kilobases. (δ -Crystallin mRNA is approximately 2.0 kilobases long^{19,21}.) Since the total length of g δ Cr-1 and g δ Cr-2 or g δ Cr-3 is about 10 kilobases, the intervening sequences amount to approximately 80% of the δ -crystallin gene.

The present data suggest that the two δ -crystallin genes are very similar, as judged by the pattern of intervening sequences in both 3' halves of the genes. The fact that the two 3' halves of the δ -crystallin genes are contained in a 4- and 12-kilobase fragment after *Eco*RI digestion indicates that the two genes have different flanking sequences at their 3' ends. It is possible that the 5' halves of both genes are present in the *Eco*RI fragment of approximate length 7.5 kilobases. This is consistent with the results obtained on the quantitation of hybridisation of ³²P-cDNA to different *Eco*RI fragments separated by electrophoresis after reverse-phase column chromatography. The relative amount of ³²P-cDNA hybridised to each δ -crystallin DNA band was estimated by scanning the autoradiogram. If one takes the amount of radioactivity hybridised to the 3-kilobase fragment as one unit, then the relative amounts of ³²P-cDNA hybridised to the 4-, 7.5- and 12-kilobase fragments were 2.1, 7.0 and 2.3 units, respectively. Thus, there was almost four times as much hybridisation to the 7.5-kilobase fragment as to either the 4- or 12-kilobase fragments. The remaining 3.0-kilobase fragment derived by *Eco*RI digestion of the chicken DNA hybridised much more weakly to the ³²P-cDNA, suggesting that it contains relatively few δ -crystallin mRNA gene sequences.

Further evidence for two δ -crystallin genes

In contrast to the results obtained with *Eco*RI, *Bam*HI digestion of chicken DNA gave two major fragments of approximately 8 and 10 kilobases which hybridised to nick-translated p δ Cr-2 DNA (Fig. 3). This is of interest since the cloned cDNA has a central *Eco*RI site but lacks a site for *Bam*HI²¹. Thus, the two

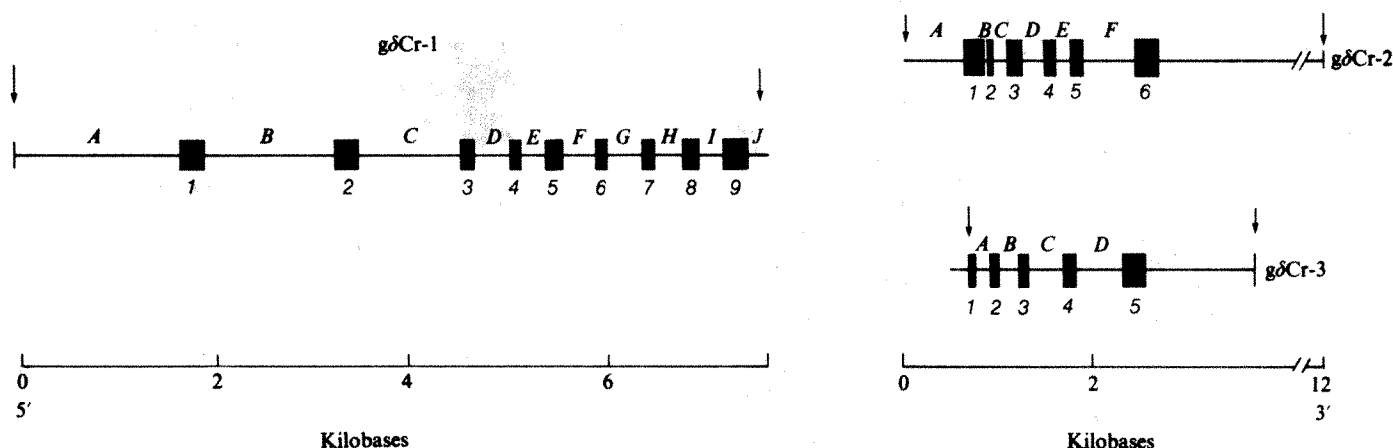


Fig. 2 Diagrammatic representation of intervening (IVS) and mRNA gene sequences (mRGS) in the cloned δ -crystallin gene fragments. Hybrid molecules of g δ Cr-1 DNA: δ -crystallin mRNA, g δ Cr-2: δ -crystallin mRNA, and g δ Cr-3: δ -crystallin mRNA (see Fig. 1) were measured, using a Numonics digitiser and statistically analysed. The orientation of the fragments was determined as given in the text. The arrows (\downarrow) indicate the *Eco*RI sites. The measured lengths (\pm s.d.) of IVS (letters) and mRGS (numbers) were: g δ Cr-1 (36 molecules): A, 1.60 ± 0.3 ; B, 1.30 ± 0.10 ; C, 1.10 ± 0.16 ; D, 0.40 ± 0.07 ; E, 0.30 ± 0.05 ; F, 0.39 ± 0.06 ; G, 0.32 ± 0.05 ; H, 0.35 ± 0.06 ; I, 0.23 ± 0.05 ; J, 0.19 ± 0.05 ; 1, 0.24 ± 0.09 ; 2, 0.24 ± 0.05 ; 3, 0.13 ± 0.03 ; 4, 0.09 ± 0.03 ; 5, 0.12 ± 0.02 ; 6, 0.11 ± 0.03 ; 7, 0.17 ± 0.04 ; 8, 0.07 ± 0.03 ; 9, 0.19 ± 0.06 . g δ Cr-2 (24 molecules): A, 0.67 ± 0.09 ; B, not measured; C, 0.28 ± 0.05 ; D, 0.23 ± 0.04 ; E, 0.23 ± 0.04 ; F, 0.55 ± 0.1 ; 1, 0.18 ± 0.04 ; 2, not measured; 3, 0.13 ± 0.04 ; 4, 0.10 ± 0.03 ; 5, 0.17 ± 0.03 ; 6, 0.24 ± 0.06 . g δ Cr-3 (23 molecules): A, 0.13 ± 0.03 ; B, 0.25 ± 0.06 ; C, 0.39 ± 0.06 ; D, 0.50 ± 0.13 ; 1, 0.06 ± 0.02 ; 2, 0.08 ± 0.02 ; 3, 0.10 ± 0.03 ; 4, 0.13 ± 0.03 ; 5, 0.24 ± 0.05 . —, IVS; ■, mRGS. The segment shown as a broken line in g δ Cr-3 was not visualised but may be present by analogy with g δ Cr-2.

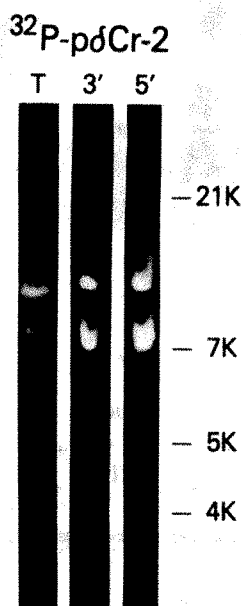


Fig. 3 Autoradiogram of a Southern blot of *Bam*HI-digested chicken DNA after hybridisation to 32 P-labelled p δ Cr-2 DNA. DNA isolated from 6-day-old chicken embryos was digested with *Bam*HI in the buffer recommended by the manufacturers (New England Biolabs), subjected to electrophoresis in 1% agarose gel, transferred to Millipore filters²³ and hybridised⁵⁷ to different 32 P-labelled probes²¹. Each sample (20 μ g) was digested with 40 enzyme units for 12 or 24 h. Digestion with addition of fresh enzyme at 2, 4 and 6 h (final substrate:enzyme ratio 1:6) did not change the hybridisation pattern. p δ Cr-2 was cut by *Eco*RI to give two fragments 4.2 kilobases and 1.3 kilobases long containing 5' and 3' sequences of the cloned cDNA insert, respectively. These two fragments were separated by electrophoresis in a 5% polyacrylamide gel and recovered by electrophoretic elution⁵⁸, concentrated by DEAE-cellulose chromatography and ethanol precipitated. Nick translation was done with 32 P-dCTP (300 Ci mmol⁻¹) according to Maniatis *et al.*⁵⁹. Each hybridisation reaction (20 ml) contained 4–6 $\times 10^6$ c.p.m. (10^7 – 10^8 c.p.m. μ g⁻¹). T = total (uncut nick-translated p δ Cr-2 DNA).

*Bam*HI fragments were not generated by cutting in the genomic regions corresponding to the cloned cDNA sequences. Unlike the cloned *Eco*RI fragments, each of the *Bam*HI fragments contained sequences complementary to the 3' and 5' halves of the cloned cDNA probe (Fig. 3). This suggests that the *Bam*HI fragment was not produced by cutting an intervening sequence within the δ -crystallin gene. The presence of two *Bam*HI fragments is consistent with the possibility that there are two separate genes for δ -crystallin. The four *Eco*RI fragments or the two *Bam*HI fragments containing δ -crystallin sequences are apparently not derived from two structural alleles of the δ -crystallin gene, since similar restriction fragments were obtained from DNA of inbred chickens (line 6, subline 3, US Dept of Agriculture, East Lansing, Michigan) and from DNA of a parthenogenetic turkey²⁹ (data not shown).

Discussion

In this investigation we have isolated, cloned and determined the pattern of intervening sequences in three δ -crystallin gene fragments produced by *Eco*RI digestion of the chicken genome. This is the first time that gene sequences for a lens crystallin gene have been examined. δ -Crystallin gene sequences are of special interest because they are expressed principally in the embryo^{3,4,10,16} and their activity can be studied *in vivo*^{4–11} and in tissue culture^{12,15}. The present study thus provides a starting point for further analysis of the regulation of δ -crystallin gene expression at the molecular level.

Our data are consistent with the existence of at least two separate non-allelic δ -crystallin genes in chickens. The fact that

*Bam*HI DNAs digested from extensively inbred chickens and a parthenogenetic turkey have similar restriction patterns for the δ -crystallin sequences to the *Bam*HI-treated DNA from the commercially available chickens (Truslow Farms) argues against interpreting our data as evidence for two structural alleles of one δ -crystallin gene. The similarity in the restriction patterns of δ -crystallin DNA sequences in turkeys with those in chickens is consistent with the similarity in the δ -crystallin protein in these two species⁸. The two δ -crystallin genes could be adjacent with a relatively small intergenic spacer, as in the human α -globin genes^{30,31}, or they may be widely separated in the genome. The data do not exclude the presence of more than two identical δ -crystallin genes which are tandemly repeated, but previous hybridisation kinetic experiments with cDNA do not support this notion²⁰.

δ -Crystallin is composed of subunits with approximate molecular weights of 50,000 and 48,000 (ref. 17). Peptide analyses have indicated that these subunits must have very similar primary structures¹⁷; only two tryptic peptide differences have been noted between the δ -crystallin subunits³². The presence of two different genes for δ -crystallin suggests that the larger and smaller subunits of this protein may be synthesised on separate mRNAs. This would be of considerable interest with respect to the control of their translation, since it is known that the ratio of synthesis of the δ -crystallin subunits is affected by the intracellular concentration of electrolytes³³. Interestingly, the rat preproinsulin gene has recently been shown to be encoded by two similar, non-allelic genes which are equally expressed^{11,34}. However, the possible existence of two genes for δ -crystallin does not necessarily mean that each subunit is encoded by a separate gene. One subunit may be derived from the other by post-translational processing³⁵, or differential splicing of transcripts from a single gene could lead to different mRNAs^{36–38}.

The present data demonstrate that the δ -crystallin genes are highly interrupted in comparison with other eukaryotic genes^{39–49}. The chicken conalbumin gene which is interrupted 17 times⁵⁰ is the only other gene reported yet that has as many or more intervening sequences as the δ -crystallin gene. If the mRNA gene sequences are separated into functional units or domains^{51,52} the large number of intervening sequences may indicate that δ -crystallin is structurally complex. Some interesting features of δ -crystallin are that it has a greater content of leucine and isoleucine than other eukaryotic structural proteins⁵³, it has a very low proportion of aromatic amino acids⁵³, and it has a high degree of α -helical structure^{54,55}. Finally, it would be particularly interesting if the unusual abundance of intervening sequences in the δ -crystallin gene was related to its distribution among animals. δ -Crystallin is confined to birds and reptiles, as judged by immunological^{6,7} and cDNA hybridisation⁸ studies. This special distribution of δ -crystallin differs from the ubiquitous distribution of other proteins, such as actin, histones or cytochrome *c*, for example, which have uninterrupted genes⁵⁶.

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Contribution of immunoglobulin heavy-chain variable-region genes to antibody diversity

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A mouse cloned cDNA probe containing a variable (V) region belonging to the V_HIII subgroup has been used in filter hybridisations to estimate the number of heavy-chain V-genes in this subgroup of mouse and human DNA. There seem to be about 10 and 20 V_H-genes hybridising to this probe in mouse and human DNA, respectively. Studies of cross-hybridisation of the related V_κ-genes from MOPC21 and MPC11 myelomas indicate that the experiments detect all members of the V_HIII subgroup.

THE antibody combining site comprises areas of the variable (V) regions of both heavy (H) and light (L) chains so that antibody diversity is a function of the germ-line diversity of both H- and L-chain genes combined with possible somatic modifications of these V-gene sequences. The regions of maximum variability occur in the three hypervariable regions which have been charted in the variability plots of Kabat¹. Each of the hypervariable regions occurs within or around the antigen binding site² and so each probably plays a part in the variability of antibodies. Recent studies of mouse L-chain genes have shown that the V segments in germ-line DNA are separate from the C-genes in the genome^{3–5}, and furthermore, that several J segments occur (equivalent to amino acid residues 96–108 (refs 6–8)) separated from the C_κ segment by a large intervening sequence. The integration of the immunoglobulin gene specifically involves fusion of one of a number of V segments with one of the J segments^{6–8} while the large intervening sequence is maintained^{4,7,8}. In the case of κ L-chain genes, residue 96 seems to be highly variable as a direct consequence of V–J joining^{7,8}. This residue, and thus, this event explains at least some of the variability of the third hypervariable region in κ-chains. In addition, it has been argued that variability of the first two κ-chain hypervariable regions and the remainder of the third hypervariable region are entirely encoded by germ-line genes⁹ whereas the λ L-chain seems to undergo some V-segment somatic variation¹⁰. There are no data for H-chain V-genes, and a first approach is to assess the variability of germ-line DNA by hybridisation experiments designed to detect the whole V_H-gene complement of a subgroup. Ideal probes for

such experiments are plasmid clones containing complementary DNA (cDNA) copies of immunoglobulin mRNA¹¹ which can be used in the filter hybridisation procedure of Southern¹². Thus, hybridisations can be carried out with suitable V_H probes and the number of hybridisation bands obtained can be equated with V-genes complementary to this probe. To assess the relevance of this number to the V-genes of a protein subgroup (that is, the group of V-region sequences bearing similar or identical framework (non-hypervariable region) amino acid residues), it is necessary to know the extent of V-gene cross-hybridisation in filter hybridisation experiments. In the present experiments, we have used a cloned cDNA probe of a mouse V_H sequence belonging to the V_HIII subgroup to determine the number of germ-line V_H-genes in this subgroup of mouse and human DNA. In the conditions used, we find that the probe hybridises to about 10 V_H-genes in mouse DNA and about 20 V_H-genes in human DNA. These numbers probably represent the size of the V_HIII subgroup in the respective DNAs, for by studying the cross-hybridisation of the κ V-gene sequences from MPC11 and MOPC21 mouse myelomas, we conclude that all members of the V_HIII subgroup should cross-hybridise.

Extent of V-gene cross-reaction in filter hybridisation

A characterisation of V-gene cross-hybridisation can be facilitated by comparing two V-genes of similar origin which differ in both the framework and hypervariable residues. The mouse κ-chains MPC11 and MOPC21 are suitable for such an analysis because protein sequencing of both chains^{13,14} shows that the V segments differ by approximately 20% of residues (excluding the J region and an additional region of 12 amino acids present

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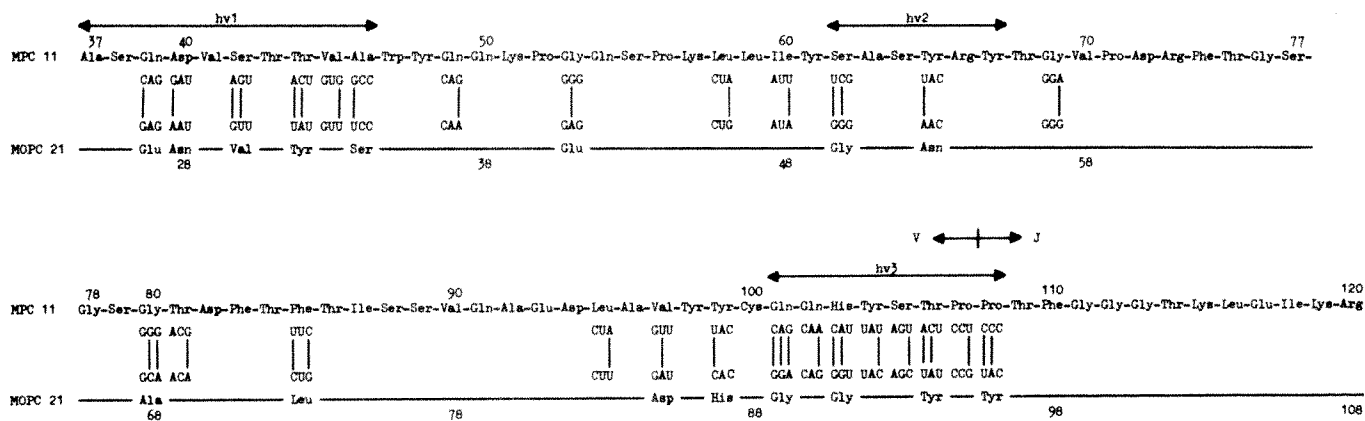


Fig. 1 Comparison of MPC11 and MOPC21 V_{κ} sequences. The nucleotide sequence of MPC11 V_{κ} shown was derived by the chemical degradation procedure¹⁶ using the 380-base pair *Hind*II fragment derived from $p\kappa(11)^{24}$ as a source of V-region material. This fragment was labelled with ³²P (using polynucleotide kinase) redigested with *Hinf* and *Hpa*II. The resulting fragments were isolated from acrylamide gels and subsequently subjected to degradation and gel analysis²⁷. Protein sequences were taken from Svasti and Milstein¹³ and Smith¹⁴. The MPC11 κ -chain is 12 amino acids longer than MOPC21 at the N terminus of the mature protein; each peptide chain is numbered here accordingly. Therefore, the J segment, for example, extends from amino acid 108 to 120 in MPC11 and 96 to 108 in MOPC21. Where a codon differs between MOPC21 and MPC11, the full codon is given and the bases which are different within the codon are indicated by a vertical line. All other codons are identical but some ambiguities in the nucleotide data remain: we have not been able to assign convincingly bases to the third residues of MPC11 codons 70 (Val) or 75 (Thr), nor to the third residue of MOPC21 codons 41 (Gln), 47 (Leu) or 77 (Ser). hv, Hypervariable region.

at the amino terminus of the MPC11 κ -chain¹⁴). We have compared the nucleotide sequences of these V-regions and the results of this comparison are shown in Fig. 1 (note that the additional N-terminal residues of MPC11 necessitate the use of an alternative numbering system in MPC11 and MOPC21 in this figure). The sequence data contained in Fig. 1 extend from the codon corresponding to amino acid 25 of MOPC21 (residue 37 on MPC11 numbering) through the V-J junction (residues 95–96 on MOPC21 numbering) to the end of the J segment (residue 108). The nucleotide sequences were obtained by primed reverse transcriptase sequencing of MOPC21 L-chain mRNA¹⁵ or the chemical degradation sequencing methodology¹⁶ using *Hind*II restriction fragments prepared from a cDNA clone of MPC11 κ -chain [$p\kappa(11)^{24}$] (see Fig. 2) containing the MPC11 V-region¹⁷. The percentage of amino acids different in the V segment of the protein sequence compared in Fig. 1 is ~23% whereas the nucleotides differ by ~18%. This proportion of difference is representative for the V-regions as the N termini of the chains (not compared in Fig. 1) also differ by about 20% of their amino acids^{13,14}.

The distribution of nucleotide differences within the two proteins is interesting from an evolutionary point of view. The germ-line progenitors of these V-genes would seem to be derived from separate V-genes which presumably had a common evolutionary origin before V-gene duplication. The framework residues of the two genes have subsequently drifted far less than the hypervariable regions and indeed, much of this drift occurs at the nucleotide level (as manifested by silent base change) but not at the amino acid level. The hypervariable regions, on the other hand, exhibit considerable variation in both amino acid changes and silent nucleotide changes. This pattern of difference between related V-genes suggests that evolutionary pressure is exerted to change the antibody combining site (the hypervariable regions) and favours the conclusion that antibody variability in mouse κ -chain results extensively from evolutionary pressures by the creation of stable V-genes rather than by extensive somatic mutation at the level of individual antibody-producing cells¹¹.

The nucleotide comparison shown in Fig. 1 indicates the type of pattern of difference we can expect between related but different V-genes. We have, therefore, considered whether such V-genes would cross-hybridise in filter hybridisation experiments using different areas of the MPC11 V-region sequence from the cDNA plasmid $p\kappa(11)^{24}$ (Figs 3, 4). Restriction enzyme mapping and nucleotide sequencing of $p\kappa(11)^{24}$ (ref. 18) and nucleotide sequence data of the C_{κ} sequence¹⁵ yield the partial restriction map of $p\kappa(11)^{24}$ shown in Fig. 2. The enzyme *Hind*II

cleaves the $p\kappa(11)^{24}$ DNA to produce, among others, a 380-base pair fragment which extends from the codon for amino acid residue 10 (using the Smith numbering¹⁴) through the whole V-region of MPC11 to the codon for amino acid 137 within the C_{κ} -region; thus, this V fragment consists of V, J and part of the C_{κ} segment. *Mbo*II cleaves this 380-base pair V fragment twice—at the codon for amino acid 96 (Smith numbering) to yield a fragment of 260 base pairs (V-region sequences only), and at the codon for amino acid 127 to give smaller fragments of about 100 base pairs (consisting of the portion of V-region containing the third hypervariable region, the J segment plus the C_{κ} segment) and about 30 base pairs (consisting of C_{κ} only).

Figure 3 shows the hybridisation of ³²P-labelled cDNA made from MOPC21 κ -chain mRNA (made with reverse transcriptase using the specific primer T₂G₃T (ref. 19)) with *Hind*II restriction fragments of $p\kappa(11)^{24}$, the isolated intact 380-base pair *Hind*II V fragment or this V fragment cleaved by *Mbo*II. The restriction digests (see Fig. 3 legend) were fractionated on a 2% agarose gel before binding to a cellulose nitrate filter¹² for the hybridisation reaction. *Hind*II-digested $p\kappa(11)^{24}$ shows two major bands of hybridisation to the heterologous MOPC21 κ cDNA (slot b), the smaller of the two components being the 380-base pair V fragment described above. The isolated V fragment (prepared by fractionation of *Hind*II digested $p\kappa(11)^{24}$ DNA on a polyacrylamide gel followed by elution of the 380-base pair fragment) electrophoresed in slot a, shows similar

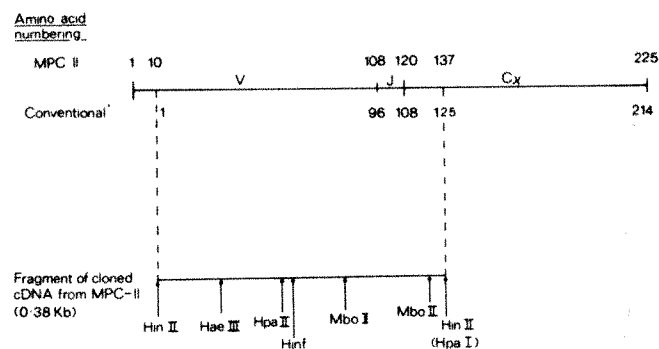


Fig. 2 Partial restriction map of the 380-base pair fragment from the MPC11 clone $p\kappa(11)^{24}$. The κ L-chain protein is depicted together with the partial restriction map of a *Hind*II fragment of $p\kappa(11)^{24}$ extending from the codon for MPC11 residue 10 to residue 137 (Smith numbering¹⁴). To relate this map to the data given in Fig. 1, the relative numbering positions of the 'conventional' (MOPC21) κ L-chain protein are written beneath the MPC11 numbering.

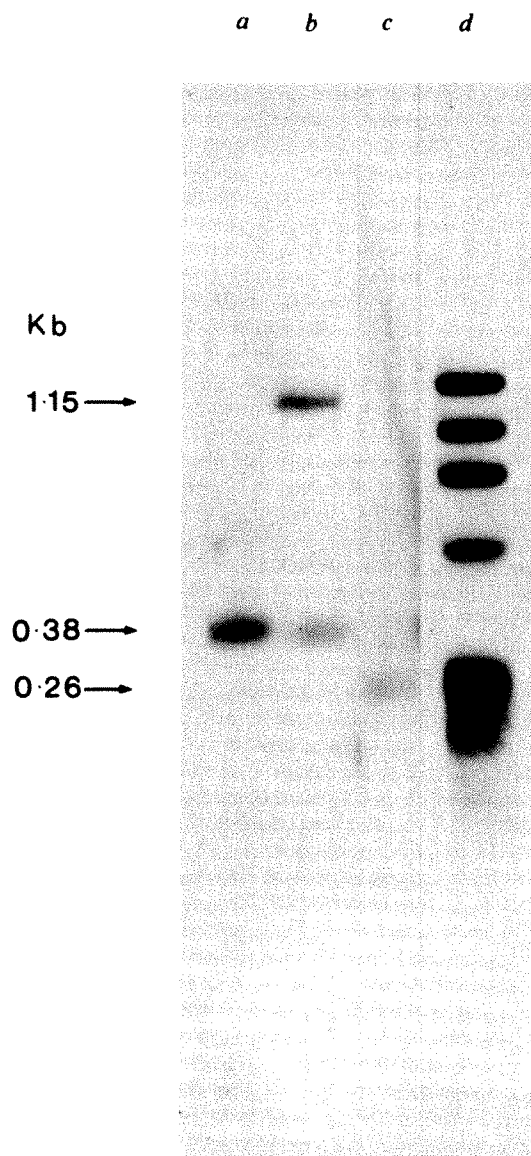


Fig. 3 Cross-hybridisation of MOPC21 κ -cDNA with *Mbo*II fragments of the isolated 380-base pair fragment from MPC11 $\rho\kappa(11)^{24}$. $\rho\kappa(11)^{24}$ DNA was digested to completion with *Hind*II, a portion fractionated on a 4% polyacrylamide gel and the 380-base pair fragment isolated¹⁸. An aliquot of this fragment was further digested with *Mbo*II. For the filter hybridisation, the digests of the various DNA samples were fractionated on a 2% agarose gel, and the DNA was denatured *in situ* and transferred to a sheet of cellulose nitrate¹² (Schleicher and Schüll BA-85). The filter was treated before hybridisation as described previously^{28,29} and hybridised for 48 h at 65 °C in 6×SSC containing 0.1% SDS, 0.2% Ficoll 400, 0.2% bovine serum albumin, 0.2% polyvinylpyrrolidone 360, 50 $\mu\text{g ml}^{-1}$ sonicated and denatured salmon sperm DNA plus 10 $\mu\text{g ml}^{-1}$ poly(rA) using 5×10^6 c.p.m. of ^{32}P -cDNA made on MOPC21 κ L-chain mRNA (by reverse transcriptase and T₂G₃T primer, specific activity of cDNA = 10^7 c.p.m. per μg (ref. 19)). After hybridisation, the filter was washed at 65 °C (30 min per wash) once with 6×SSC containing all the ingredients of the hybridisation except ^{32}P -cDNA, followed by five washes of 1×SSC and 0.1% SDS, after which the filter was rinsed in 2×SSC, dried and autoradiography carried out overnight using prefogged X-ray film³⁰. DNA samples fractionated on agarose and hybridised: a, isolated 380-base pair *Hind*II fragment of $\rho\kappa(11)^{24}$ (4 ng); b, complete *Hind*II digest of $\rho\kappa(11)^{24}$ (10 ng); c, *Mbo*II digest of the isolated 380-base pair *Hind*II fragment (10 ng); d, *Hae*III-digested ϕX174 DNA, labelled with ^{32}P before electrophoresis to serve as internal size standards in the DNA fractionation and transfer. kb, kilobase.

hybridisation to this region. On the other hand, the 380-base pair V fragment displayed a new, more rapidly migrating hybridisation component (260 base pairs) when cleaved with *Mbo*II. This component corresponds to the V segment of MPC11 (from which J and C_κ segments are absent) cross-hybridising to MOPC21 V-region cDNA. (The 120-base pair fragment also resulting from *Mbo*II cleavage of the 380-base pair V fragments

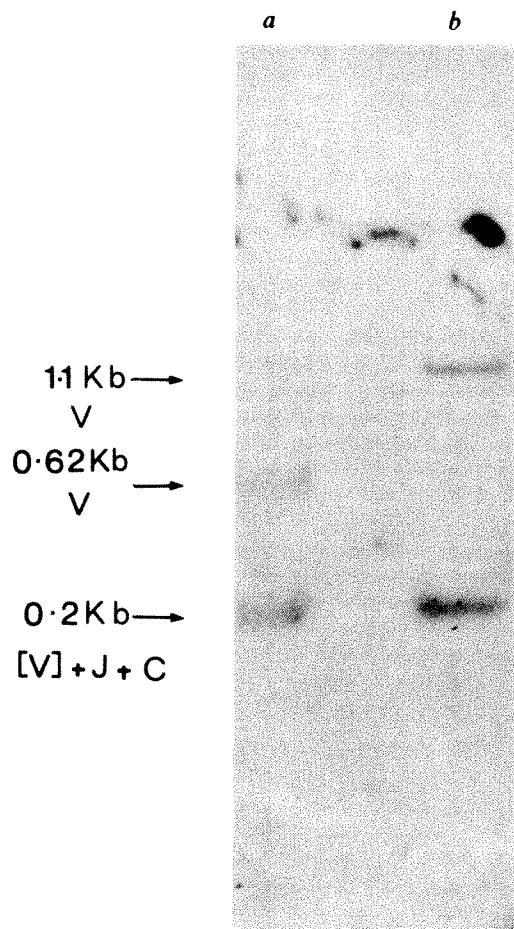


Fig. 4 Cross-hybridisation of MOPC21 κ -cDNA with *Hinf* and *Hpa*II digests of the isolated 3.1-kilobase *Hpa*I fragment from $\rho\kappa(11)^{24}$. $\rho\kappa(11)^{24}$ DNA was cleaved by *Hpa*I, fractionated on 0.8% agarose and the 3.1-kilobase fragment eluted from the gel⁴. Aliquots (20 ng) of this fragment (containing V, J and part of the C) were redigested with either *Hinf* (slot a) or *Hpa*II (slot b) and these digests were fractionated on a 2% agarose gel, transferred to a cellulose nitrate filter¹² and hybridised to 3×10^6 c.p.m. MOPC21 ^{32}P - κ L-chain cDNA as described in Fig. 3 legend. Sizes shown in kilobases (kb) were determined relative to unlabelled ϕX174 DNA (digested with *Hae*III) which was visualised after staining with ethidium bromide.

(which includes J and C_κ segments) does not show in the hybridisation because it was too small to bind effectively to the cellulose nitrate filter.)

Thus, the two V segments (MOPC21 and MPC11) do cross-hybridise in the conditions used for these experiments and we can, therefore, reasonably conclude that V-genes up to about 18% different at the nucleotide level will cross-hybridise. Interestingly, the MPC11 and MOPC21 V sequences from the 5' side of the *Mbo*II cleavage site (between Ala-Val position 97 (ref. 14)) contain only two substantial regions of perfect homology, one 29 bases long (Thr-86 to Leu-95) and the other 31 bases long (Val-70 to Gly-80). These areas represent the best possibilities for cross-hybridisation of the V-genes. The remainder of the sequences are broken up by base differences of various complexity. In view of this, we examined further the V-gene cross-hybridisation with segments of the V-region which exclude the homologous stretches of 29 and 31 nucleotides.

Restriction mapping of $\rho\kappa(11)^{24}$ showed the presence of sites for the restriction enzyme *Hinf* between the codons for Gly-Val at positions 69–70 and *Hpa*II between Tyr and Arg at positions 65–66 (Fig. 2). Thus, by cleaving $\rho\kappa(11)^{24}$ with either of these enzymes it is possible to generate a V segment suitable for cross-hybridisation studies which excludes the relatively long stretches of homologous sequence but includes the second hypervariable region. To simplify the analysis of the cross-hybridisation of these V segments, we prepared a subfragment of $\rho\kappa(11)^{24}$ with which to carry out the intra-V-segment

cleavage. The nucleotide sequence of the C_{κ} -region showed a cleavage site for *HpaI* at the codon for amino acid 125 (ref. 15). *HpaI* cleaves $p\kappa(11)^{24}$ at this site and at a site in the pMB9 vector to yield two large fragments of 3.7 and 3.1 kilobases. The 3.1-kilobase fragment contains the V segment together with the J and the 5' end of the C_{κ} . When this isolated 3.1-kilobase fragment is digested with *Hinf* or *HpaII*, the V segment is split into two fragments of 0.61 and 0.2 kilobases with *Hinf* cleavage and 1.1 and 0.2 kilobases with *HpaII* cleavage (unpublished data). In both cases, the 0.2-kilobase fragment is the *HpaI*-*Hinf* or *HpaI*-*HpaII* fragment containing the 5' end of the C_{κ} -region, the J plus the 3' end of the V segment (Fig. 2). The larger of the two fragments then consists of the 5' end of the V-region starting at around the second hypervariable region.

Figure 4 shows the hybridisation of purified MOPC21 L-chain cDNA with the *Hinf* or *HpaII* digests of the 3.1-kilobase *HpaI* fragment of the MPC11 clone $p\kappa(11)^{24}$. In both cases the large and small V segment-containing fragments hybridised to the MOPC21 cDNA. When the *HpaI* fragment was cleaved by *Hinf* we observed that both the 0.2- and 0.62-kilobase fragments hybridised the MOPC21 cDNA (slot a) and similarly the 0.2- and 1.1-kilobase fragments of the *HpaII* digest hybridised to the MOPC21 cDNA (slot b). Thus, again we observe that the fragment of the MPC11 clone which contains the V segment hybridised to the heterologous MOPC21 cDNA. In addition, in this case, we detect hybridisation between the V segments where no more than 18 uninterrupted base pairs can form. (For example, in the area of nucleotide sequence shown in Fig. 1, 15 homologous base pairs can form between codons for Gln-54 and Leu-58 whereas in the region where only protein sequence is known a possible 18-base pair region could exist between Ser-26 and Val-31.) The results of these cross-hybridisation experiments show that V_{κ} -genes with only short areas of homologous base sequence, interrupted extensively by mismatches, do cross-hybridise in the filter hybridisation experiments. It seems reasonable to assume that this situation will also occur with all related V-genes as the patterns of variability in general follow that shown by MPC11 and MOPC21 V_{κ} -regions. These cross-hybridisation experiments therefore set a criterion for the extent of V-gene detection and we can say that genes differing by at least 18% of their nucleotides can be detected in this type of experiment.

It must be considered, however, that the detection of cross-hybridising V segments when using cloned DNA, need not necessarily be related to such cross-hybridisations in genomic DNA. The experiments described in Figs 3 and 4 used 0.5 ng, 2 ng and 4 ng of specific hybridising sequence compared with the expected quantity of about 0.02 ng of a particular V-gene in a genomic hybridisation. This means that we are using between 25 and 200 times as much cloned V segment in these controls as

used in the genomic blots described below. This could lead to underestimates of V-gene numbers due to lower sensitivity of the latter. However, it is also true that the sensitivity of detection of the small DNA fragments described in Figs 3 and 4 is considerably weakened (compared with that of large fragments) by the relative inefficiency of binding small fragments to the cellulose nitrate filters. On balance, the relatively high quantities of the small cloned fragments are likely to mimic the situation in the genomic hybridisations.

The number of V_H -genes in mouse and human DNA

When assaying for V-genes in genomic DNA by filter hybridisation, the genes detected must represent either a group of closely related V-genes separated from the next set by at least 18% nucleotide difference, or V-genes gradually diverging in sequence from one another up to the extent of or greater than about 18% difference. Whichever of these situations exist, the results obtained for the DNA of a given species allows an assessment of the size of the inherited V-gene pool and in turn the potential requirement for somatic variation of the V-genes. We have attempted to derive information relating to V_H -gene complexity in mouse and human DNA using a mouse cDNA plasmid probe containing V_H -gene sequences. A cDNA plasmid ($p\mu/107$) has been derived from μ H-chain mRNA (of the hybrid cell line Sp1/HL (ref. 20)) and this plasmid has been shown to contain the entire V_H and C_{μ} sequences²¹. The nucleotide sequence of the 5' end of the V_H segment of $p\mu/107$ has been derived and is shown in Fig. 5 (extending from amino acid codons 1 to 38). This V_H -region (an anti-sheep red blood cell antibody) can be assigned to the mouse V_{HIII} subgroup, from which it differs by two amino acids in the first framework region from the basic V_{HIII} sequence¹ and a further four residues in the first hypervariable region (16% difference).

As shown in Fig. 5, the other two well-characterised mouse V_H subgroups [V_{HI} and V_{HV} (ref. 1)] differ extensively from the anti-sheep red blood cell Sp1/HL V_H sequence. V_{HI} differs by about 52% and V_{HV} by about 42% from mouse V_{HIII} . By considering the cross-hybridisation capabilities of V-genes analysed in the previous section, we can see that the V_H sequence in $p\mu/107$ would be expected to cross-hybridise to all V_{HIII} members because related V_{HIII} genes are likely to display the same type of variability at the nucleotide level as seen for the related V_{κ} sequences¹. The V_H -genes would be able, therefore, to cross-hybridise by virtue of relatively small regions of uninterrupted base pairing. On the other hand, comparison of the $p\mu/107$ V-region with the other subgroup sequences indicates that cross-hybridisation is unlikely because, on the basis of the sequences shown we can only find one region of potentially 15

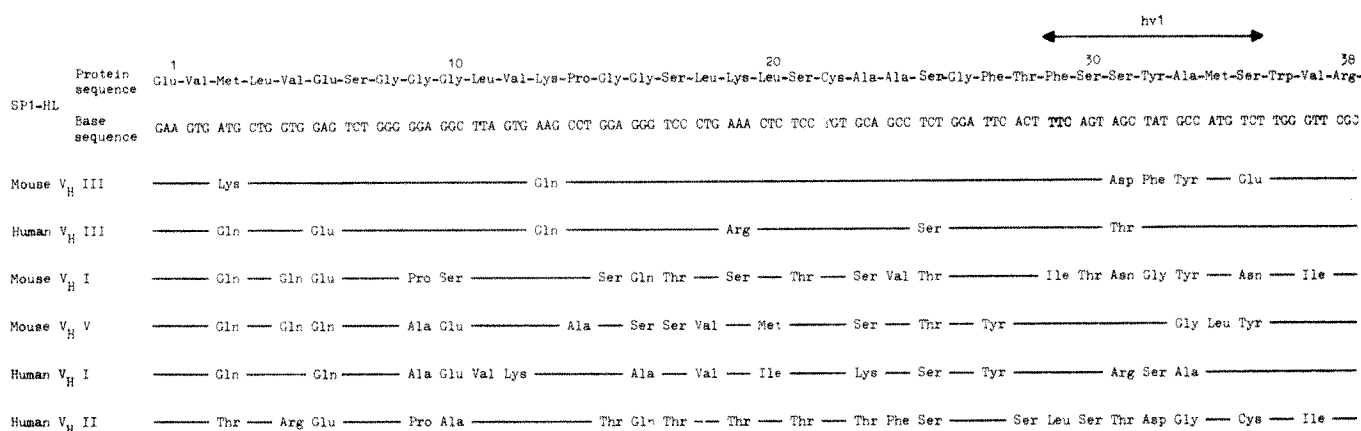


Fig. 5 Sequence of 5' terminus of the Sp1/HL heavy-chain anti-SRBC specificity and comparison with mouse and human V_H subgroups. The nucleotide sequence shown for Sp1/HL was derived by chemical degradation procedures of Maxam and Gilbert¹⁶ using an *EcoRI*-*HpaII* restriction fragment from $p\mu/107$ (ref. 21). The derived protein sequence is compared with the basic sequences of characterised mouse and human V_H subgroups¹.

homologous base pairs (in V_HV). We have also compared the Sp1/HL sequence with the human V_H subgroup basic sequences (Fig. 5). This comparison revealed that the Sp1/HL sequence is also very similar to human V_{HIII} (about 16% difference between residues 1 and 38) and, further, is more similar to the Sp1/HL sequence than the basic V_{HIII} of mouse in the area designated as the first hypervariable region. Thus, we should expect to achieve intra-subgroup cross-hybridisation between the mouse V_{HIII} probe and the human V_{HIII} genes, whereas the other human V_H subgroups differ to the extent that little cross-hybridisation would be expected (for example, there is about 39% difference between the Sp1/HL V sequence and human V_{HI} and 55% between Sp1/HL V_H and human V_{HII}).

Therefore, by carrying out filter hybridisation of mouse and human DNA with the $p\mu/107$ probe, we can estimate the V_H -gene complement for the V_{HIII} subgroup in these species. Figure 6 shows the result of filter hybridisation between ^{32}P -labelled $p\mu/107$ and fractionations of *Eco*RI-digested BALB/c mouse liver DNA (slot *a*) and *Eco*RI-digested human placental DNA (slot *b*). The hybridisation of ^{32}P -labelled $p\mu/107$ to mouse liver DNA yielded around 10 bands of hybridisation ranging from about 12.5 kilobases to about 2.7 kilobases. We have identified the largest hybridising band (12.5 kilobases) as containing the C_μ -gene so that the remaining bands represent V_H -gene hybridisation (unpublished results). In addition, it seems likely that each band represents only one hybridisation band rather than co-electrophoreses of fragments of similar size, as digests of mouse DNA with different enzymes yielded a similar number of hybridising restriction fragments. Previous studies of mouse V_κ -genes has indicated that, in general, V_κ -genes are at least 10 kilobases apart²², and our own studies on isolated V_H -genes from human DNA indicates that the average spacing of V_H -genes is greater than 10 kilobases (unpublished data). It is, therefore, a reasonable conclusion that each band contains a single V_H -gene. Thus, there are only about 10 V_H sequences in the mouse germ-line DNA which fall within the detection range of 18% nucleotide difference, and the mouse V_{HIII} subgroup therefore seems to contain only about 10 V_H -genes. Protein sequence data indicate that only four or five V_H subgroups occur in mouse¹ and assuming that they are all of a similar size, we expect about 40–50 V_H -genes in total. This number compares with the projected V_κ -gene pool size of 300 predicted from a similar type of hybridisation experiment¹¹.

Figure 6 also shows the result of hybridising the $p\mu/107$ probe to *Eco*RI-digested human placental DNA. Here we observed a more complex pattern than with mouse DNA, the hybridisation profile showing around 20 hybridisation bands ranging from about 17 kilobases to about 1 kilobase. Two of these bands hybridise to a C_μ probe¹⁸, so we are observing about 18 hybridising V_H -genes (equating each hybridising component with individual V_H -genes by analogous arguments to those for the mouse). Therefore, as we have argued that we can detect all or most V_H -genes in the human V_{HIII} subgroup, this subgroup would seem to consist of around 20 V -genes. The V_H -gene pool of human DNA would therefore seem to consist of around 80 V -genes as protein data show that there are four human V_H subgroups¹. The projected V_H -gene pool sizes calculated from the hybridisation data assume that all the protein subgroups have been discovered and that each subgroup is of approximately equal size. Discrepancies in these two parameters will, of course, introduce errors into the final value. However, study of available protein data suggests that the V_{HIII} subgroup may be a major representative of the germ-line component of V_H -genes¹.

Origin of variability of heavy-chain V-genes

The hybridisation data presented here suggest that the V_{HIII} subgroup of mouse and human DNA contains a low number of genes and that the V_H -gene pool is of the order of 40–80 genes in both species. The C_μ -gene, $C\gamma 2b$ and $C\gamma 2a$ (refs 18, 23, 26) and $C\gamma 1$ (ref. 24) genes, on the other hand, seem to represent unique components in the genome. Therefore, two clear conclusions

can be drawn. First, the V_H -gene pool must lie in the germ-line DNA separate from the C_H -gene array as in the case of the L-chains^{3–5}. Second, in order to express a V_H -gene in combination initially with the C_μ -gene, a translocation recombination event must occur to form the active H-chain gene transcription unit.

Antibody variability results from a combination of H- and L-chains and therefore the variability ultimately attained is dictated by the germ-line complement of V_H and V_L . The mouse and human V_{HIII} subgroups seem to be approximately the same size although the human subgroup is slightly larger than the corresponding group of mouse. The combination of V_H and V_κ segments in mouse could potentially generate 1.5×10^4 antibody specificities. We have no data on the V_κ complement of human DNA, but it seems likely from the available protein data that the number of κ V-genes will be smaller in human than mouse. The combination of the V_H -gene pool potential with V_κ -genes in human would, therefore, seem to be lower than the correspond-

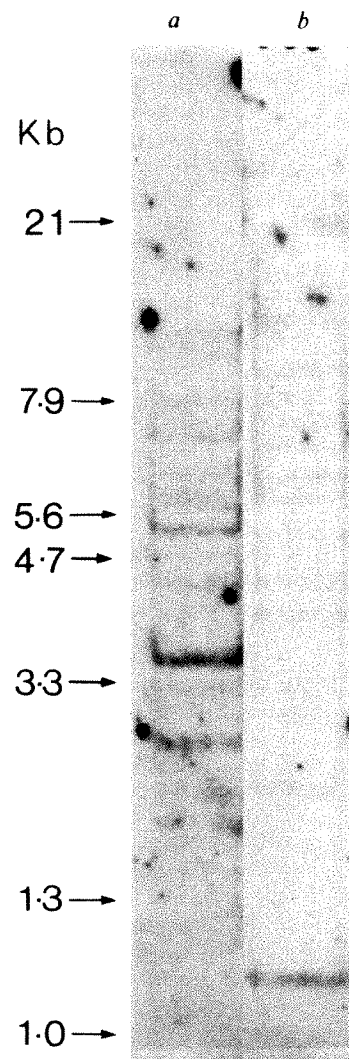


Fig. 6 Filter hybridisation patterns of the mouse heavy-chain cDNA probe $p\mu/107$ with *Eco*RI-digested BALB/c mouse liver DNA (slot *a*) and human placental DNA (slot *b*). 15 μ g of mouse liver or human placental DNA were completely digested with *Eco*RI and fractionated on an 0.8% agarose gel alongside a mixture of *Eco*RI digest of λ phage DNA (size range 21–3.3 kilobases) and *Hae*III-digested Φ X174 (fragments visible were 1.3 and 1.0 kilobases). The positions of these markers were visualised under UV light after staining with ethidium bromide and the positions are indicated on the left hand side. After staining, the DNA was transferred to a cellulose nitrate filter¹² and the filter hybridised (as described in Fig. 3 legend) for 48 h with 25 ng ml⁻¹ ^{32}P -labelled $p\mu/107$ (labelled by nick-translation^{31,32} to a specific activity of 5×10^7 c.p.m. per μ g), followed by post-hybridisation washing and autoradiography as Fig. 3.

ing combination in mouse. This would imply that somatic variation of V segments (both V_H and V_κ) has a more important role in the generation of diversity in V segments in the human species than in the mouse. In addition, the apparently lower number of V_H -genes in mouse germ line compared with the corresponding V_κ -gene number, indicates that somatic variation of V_H -genes may be more significant than in V_κ -genes of mouse.

It is possible, however, that, particularly in mouse, the $H \times L$ combination can generate all required antibodies without significant somatic variation in the V segments. It has been postulated that the single residue 96, which is involved in the translocation joining of V_κ and J_κ , may be highly variable as a result of this joining^{7,8}, so that a somatically produced hyper-variation is believed to occur in κ -chains of mouse. It is not known whether J_H segments exist in mouse or human, although some evidence for their existence has been proposed²⁵. Therefore, the effective number of V_H -regions randomly attainable

will be the product of V_H and putative J_H segments, assuming the V and J can fuse randomly. In this respect, it is interesting that the third hypervariable ($h\nu_3$) region may be largely contained in the J_H -region, unlike the analogous region of κ -chains which does not contain much of $h\nu_3$ (refs 7, 8). Therefore, the overall number of different H-chain V-regions which can be produced will depend very much on the number of J_H segments available, as the fusion of V_H with J_H would combine the first two $h\nu$ regions with the different $h\nu_3$ regions within the J_H . In addition, the sequence of Sp1/HL V-region shows that it differs significantly from the basic V_H III sequence in both framework residues and the first hypervariable region, implying that some somatic variation may be operating on these sequences.

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LETTERS

Spectrum of the Venus day sky

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The spectrum of solar radiation reflected by Venus' atmosphere has long been studied by ground-based telescopes: these studies were a major source of information on the composition of atmosphere gases and also about the upper part of the cloud layer. In December 1978 the Soviet descenders Venera 11 and Venera 12 measured the spectrum of scattered solar radiation within the atmosphere with a resolution sufficient to identify the absorption bands of atmosphere gases. We report here the first observations of the spectra of the Venus day sky.

Both vehicles landed in the equatorial region of the planet; the solar zenith angle was about 20°. The coordinates and other details of the landing area are given elsewhere¹. The results mentioned below were obtained using scanning spectrophotometers on-board the descenders. Each instrument had two measuring channels. Channel 1 recorded the spectrum of scattered solar radiation in the range 0.43–1.17 μm . The analysing element was a circular variable interference filter with a resolution of $\lambda/\Delta\lambda \approx 30$; the radiation was received from the zenith. During the station descent about 300 spectra were obtained between 62 km and the surface. Channel 2 measured the distribution of the radiation intensity in the vertical plane in four spectra regions: 0.45–0.55, 0.56–0.68, 0.9–1.14, and 1.15–1.55 μm separated by glass filters. The field of view of both channels was 20° at the 0.5 level. The instrument and its

operating conditions are described in more detail elsewhere². Figure 1 shows the spectra recorded by the Venera 11 spectrophotometer; also shown, for comparison, is the solar radiation spectrum.

The spectra at 62, 56 and 51 km were obtained inside the cloud layer. The intensities of these spectra diminish rapidly as the altitude decreases. After the vehicle emerges from the clouds (at ~49 km) the intensity varies very weakly down to 38 km and then the rapid decrease of intensity is observed as the atmosphere optical depth increases. The absorption bands of CO_2 and H_2O are seen in the spectra. The spectra measured by the two vehicles are in good agreement.

To estimate the integral flux F on the surface, the integration was made over the wavelength range 0.43–1.17 μm and over the entire upper hemisphere. It was found that $F = 72 \text{ W m}^{-2}$, which is ~3% of the solar radiation flux outside the atmosphere. The surface illuminance is about 9,000 lx, which is in good agreement with the results from Venera 9 and Venera 10 (ref. 3). According to the spectrum obtained, the colour of the surface is orange.

Figure 2 gives the height dependence of radiation intensities from the zenith for $\lambda = 0.45, 0.5, 0.635, 0.72$ and $1.0 \mu\text{m}$. The part of the curve for $\lambda = 0.5 \mu\text{m}$ is shown on a larger scale, together with experimental points. Examination of the curves shows three sublayers with different extinction coefficients: a 49–52 km sublayer is the most dense optically, 52–58 km is the most transparent and 58–62 km forms a layer with an intermediate optical density. Nearly the same stratification was found in the optical experiments on Pioneer Venus^{4–6}.

Estimates of the multiple light scattering were used to analyse the data. In one of the analyses the atmosphere was divided into 20 layers, intensities were calculated by the adding method⁷. The reflection and transmission of each layer were determined

by a two-flux approximation. The profiles of intensity, close to those measured in the continuous spectrum, were estimated using the variations layer model parameters. The following model was constructed from these results. (1) The main cloud layer is at 49–68 km, its full optical depth is $25 < \tau < 35$ in a spectral region of $0.63 < \lambda < 1.00 \mu\text{m}$; (2) the optical depth of the lower most dense part of the main cloud layer (49–52 km) is about 12; (3) the single scattering cloud particles albedo is $0.998 < a < 1.000$; (4) at 49–32 km there is very weak aerosol haze with an optical depth of about 0.7 below the main cloud layer. Optical depth has been estimated using the assumption that the scattering is anisotropic and Henyey–Greenstein phase function parameter is $g = 0.7$ (this corresponds to size of particles $\sim 1 \mu\text{m}$).

The above mentioned characteristics of the cloud layer, together with the data on the Rayleigh scattering and the absorption coefficients of H_2O and CO_2 , were used to calculate synthetic spectra in the region of the absorption bands. Comparison of synthetic and measured spectra yields a preliminary conclusion that the mixing ratio is $[\text{H}_2\text{O}]/[\text{CO}_2] \approx 2 \times 10^{-5}$ near the surface but that it gradually increases and in the clouds it is $[\text{H}_2\text{O}]/[\text{CO}_2] \approx 2 \times 10^{-4}$. The last evaluation is close to the results obtained on Venera 9 and Venera 10 (ref. 8). Both estimates strongly contradict the published results of measurements of the gas chromatograph on Pioneer Venus⁹, which give the abundance of H_2O from 0.1 to 0.5%. The gas chromatograph on Venera 12 gave the upper limit of $\text{H}_2\text{O} < 10^{-4}$ for heights under 42 km that agrees with our estimates. The decrease of H_2O mixing ratio between the clouds and the surface may be associated with some processes of chemical binding of H_2O in rocks¹¹.

The depression in the blue part of the measured spectrum (heights under 30 km, Fig. 1) can be only partially explained by the Rayleigh scattering. Sulphur in the gaseous phase (S_2) can absorb in this region and in this case the mixing ratio of $[\text{S}_2]/[\text{CO}_2]$ is $\sim 10^{-8}$. In any case this value can be considered as an approximate upper limit. There may possibly be a contribution from absorption of other molecules here, such as Cl_2 , Br_2 and NO_2 . The corresponding upper limits are approximately 10^{-8} , 10^{-10} and 5×10^{-10} . The sensitivity of the optical spectroscopy for some substances (even for low resolutions) in the Venus low atmosphere turns out to be higher than for the mass

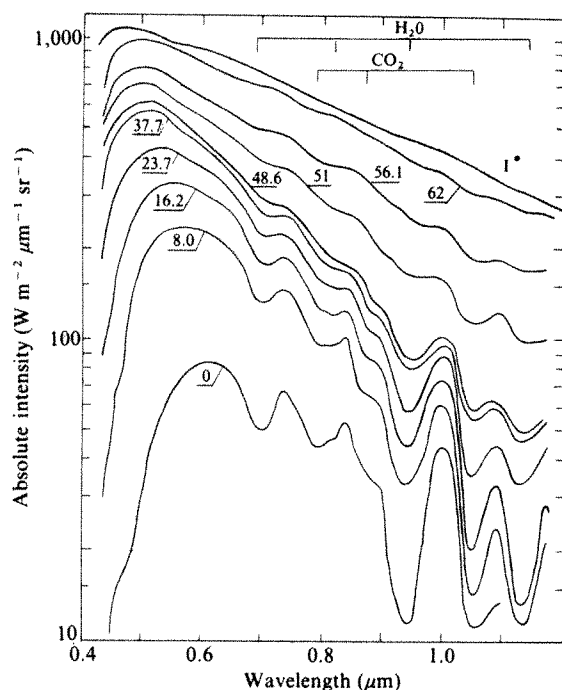


Fig. 1 Samples of spectra of scattered solar radiation obtained by Venera 11. Numbers near the curves are heights above the surface.

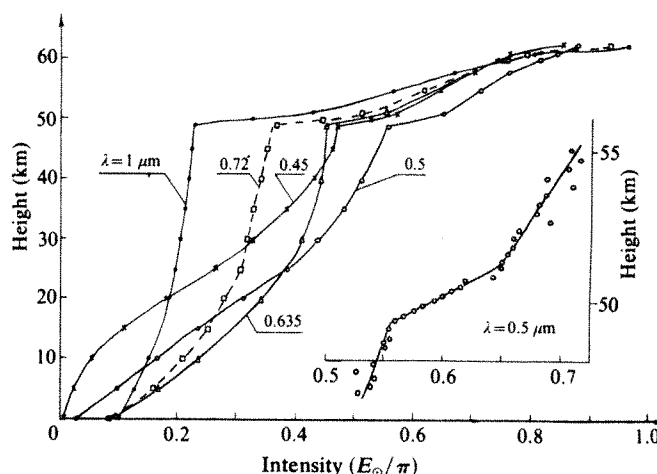


Fig. 2 Zenith intensity of the radiation as a height function for some wavelengths. E_\odot is the solar illuminance at the upper boundary of the Venus atmosphere. Numbers near the curves are the wavelengths.

spectrometer and the gas chromatograph. There is an indication that the concentration of the substance absorbing in the blue might decrease near the surface. In the altitude range 0–7 km the height dependence of intensity (in the blue) can be explained by assuming that there is only the Rayleigh scattering in this region of the atmosphere; the absorption appears only at higher altitudes. This absorption practically disappears again above 30 km. There is also some blue absorption in the top part of cloud. This cloud absorption, taking the assumption¹² into account, is caused by condensed sulphur.

More detailed reports on the above analysis of the spectra obtained on Venera 11 and Venera 12 probes are given elsewhere^{2,13,14}.

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Discovery of frequent lightning discharges in clouds on Venus

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The analysis of the composition of the Venus atmosphere made by seven Soviet Venera-type spacecraft between 1967 and 1975 has resulted in speculation about the possible role of lightning in the formation of some minor atmospheric components similar to the Earth's atmosphere. The glowing of the Venus nightside, the ashen light, sometimes observed¹ might also be explained by lightning in the Venus atmosphere. Calculations show, however, that there must be many lightning discharges to be visible from the Earth. Nevertheless, although some speculation has arisen about the existence of lightning in the atmospheres of the other planets until recently only lightning discharges in the Earth atmosphere were known.

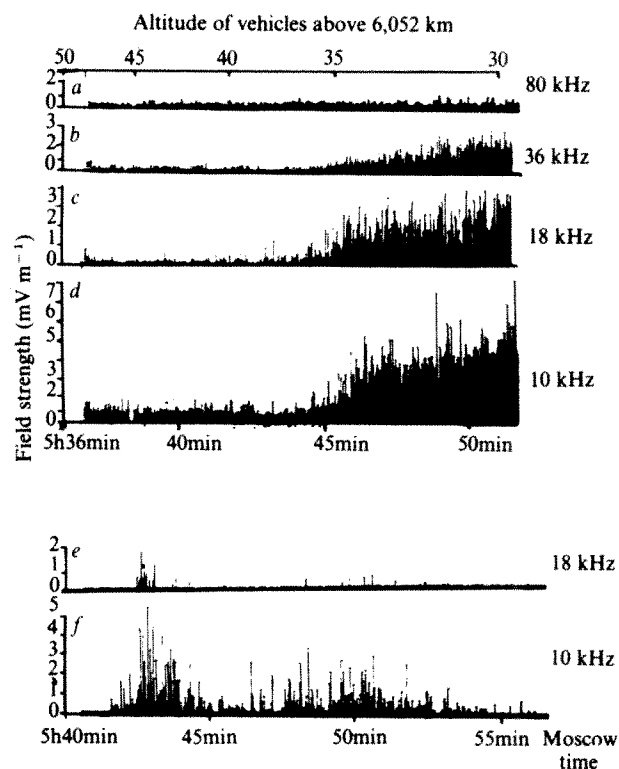


Fig. 1 Comparison of the electric thunderstorm activity of the Venus atmosphere during the Venera 12 descent (*e, f*, 21 December 1978) and the Venera 11 descent (*a-d*, 25 December 1978). The altitude scale refers to both vehicles. The signal was recorded in the bands: 1.6, 2.6, 4.6 and 15 kHz at frequencies: 10, 18, 36 and 80 kHz, respectively.

The GROZA experiment was carried out on board the Venera 11 and Venera 12 spacecraft in December 1978 to search for lightning in the Venus atmosphere². (Hara³ has developed the same idea independently, while our experiment was under preparation.) The GROZA instrument recorded impulses of the electromagnetic radiation produced by the lightning discharges. It consists of a radio receiver/spectroanalyser covering a range of 8 to 90 kHz and an impulse counter of discharge rate. The instrument had an external magnetic loop antenna operating at temperatures up to 500 °C and pressures up to 100 bar. The instrument worked both during its descent and on the planetary surface.

The following points were considered during preparation of the experiment. The intensive motions in the planetary cloud layer⁴ at heights 50–70 km were expected to contribute greatly to the accumulation of rather strong electrical charges in

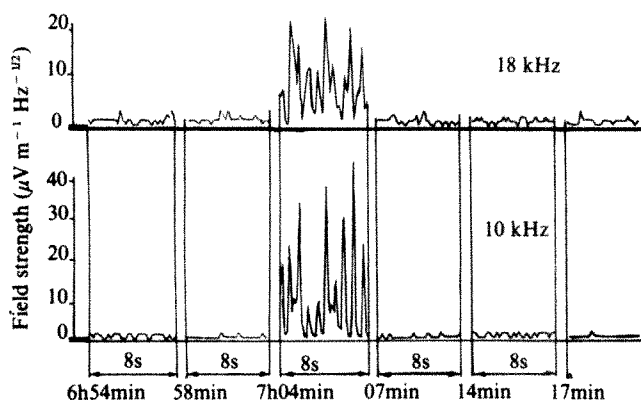


Fig. 2 The unique group of impulses recorded on the planetary surface by Venera 12, 30 min after the landing.

clouds⁵. The large altitude of the lower boundary of the cloud layer means that the discharge to the surface would require enormous potentials. 'Cloud-cloud' discharges, well known in case of the Earth are more probable. The discharges of Earth lightning are characterised by high energies of $\sim 10^9$ J. Thus, energy is determined by the conditions in which electrical discharges occur in a gas: the energy an electron obtained during its mean free path has to exceed the gas ionisation potential (that is, of 13.8 eV for CO₂ and somewhat less for air). To satisfy this condition the electric field must reach some hundreds of kV m^{-1} . Therefore, the charges accumulating in the atmosphere reach tens of coulombs⁶. The lightning is followed by the transport of a very significant charge, equal to about several coulombs, that produces the high energies of lightnings. As for aerosols their conductivity affects discharge only slightly. Hence if there is an accumulation of charges in clouds on Venus the lightning energy must be of the same order as that on the Earth.

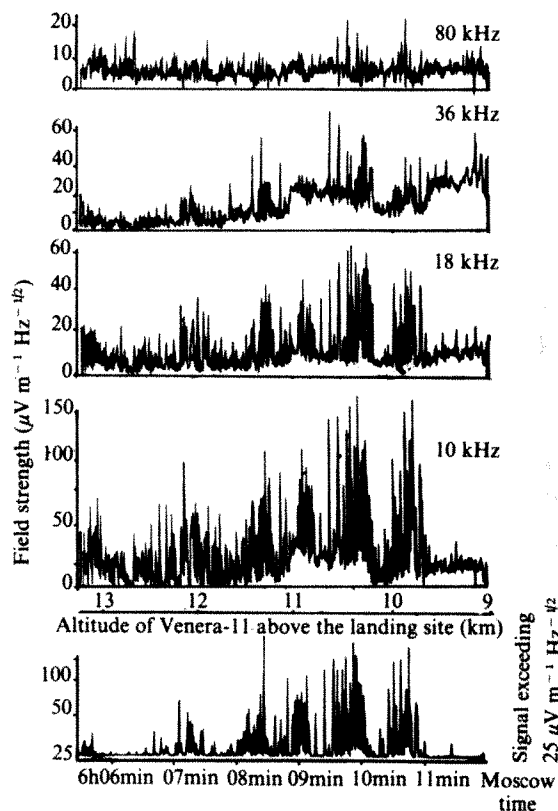


Fig. 3 Periodic sequence of the radionoise bursts recorded by the Venera 11 at heights 13–10 km. The source was the thunderstorm region with the lateral extent of ~ 150 km at $\sim 1,500$ km from the landing site.

The space vehicles had almost the same descending trajectory, and the descent operation was carried out in the same equatorial region of Venus⁷. During the descent a thousand impulses were recorded that were similar to the radiation of lightning in distant thunderstorms on Earth but with a high rate of discharge. While the Venera 11 was descending, the thunderstorm events were rather intense (on 25 December 1978) but during the descent of the Venera 12 (on 21 December 1978) there were fewer discharges. The solar activity during the latter half of December was very low and comparatively constant. The difference in the character of these events probably relates to the local nature of Venus thunderstorms. The discharge rate was 20–30 s^{-1} and in some cases reached up to 50 s^{-1} . The comparison of the records made at heights of 30–50 km for both vehicles is shown in Fig. 1: *e, f*, Venera 12; and *a-d*, Venera 11. The field strength reaches 300 $\mu\text{V m}^{-1} \text{Hz}^{-1/2}$ at 10 kHz. The

decrease of the strength with a frequency is close to f^{-1} on average. Due to the small time constant of the spectral bands (0.24 s) and the compressed character of the curves, each impulse record is in the form of vertical bars. Venera 11 recorded impulses continuously; five different sources (or events) can apparently be singled out. On the planetary surface the GROZA counters recorded during 110 min only one group of 160 impulses received for 8 s. Due to the sampling frequency (3 s^{-1}) the field strength at the 10 and 18 kHz bands resembled that shown in Fig. 2.

The sequence of impulses with noticeable periodicity associated with both the vehicle slow rotation and the directional pattern of the GROZA antenna turned out to be a key for determining some characteristics of venusian thunderstorms (Fig. 3). The sequence consists of six periods of great bursts with some hundreds of impulses in each. Calculations² showed that the signal was emitted by the source with small angular size of $\sim 5^\circ$. The field strength showed that the source was $\sim 1,500 \text{ km}$ away and had a lateral extent of about 150 km (it was assumed that the source strength in Venus clouds was similar to that in terrestrial clouds). Probably the source was a part of the cloud layer. There were about 25 lightnings per second in this source: this burst took place on the radio horizon and for the next few minutes the source should be behind the radio horizon—a fact which should have been confirmed by Fig. 3. However, more precise calculations using the diffraction theory showed that the radio occultation should have lasted for many minutes in spite of the rapid descent of the vehicle; therefore, it is difficult to explain the sudden cutoff of the signal at 6 h 11 min. Perhaps there are peculiarities in the wave propagation, typical of Venus, that we don't know about, as the field strength from other radio sources also decreases very quickly for 2–3 min.

From 8 km above the surface until they landed both vehicles recorded the many impulses having moderate amplitudes which decreased up to the same level as the radionoise near the surface (Fig. 4). This is the only event seen at the same altitude on the records of both 21 December and 25 December. In Fig. 4 the

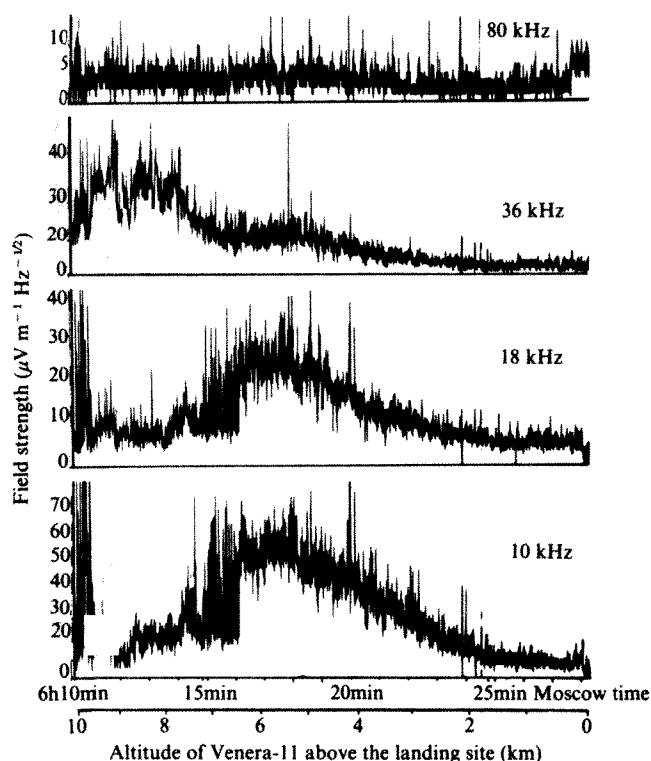


Fig. 4 Bursts recorded by the Venera 11 before the landing. It covers the altitudes 8–2 km. Immediately before the landing and on the surface the level of atmospheric radionoise was very low. The burst shows most prominence at 36 kHz.

group of impulses with distinct frequency properties can be singled out: from 6 h 11 min to 6 h 14 min the field strength was $50 \mu\text{V m}^{-1} \text{ Hz}^{-1/2}$ only in the range 33–38 kHz whereas at frequencies 10 and 18 kHz the field strength was only 10–20 $\mu\text{V m}^{-1} \text{ Hz}^{-1/2}$ —this event also remains obscure.

From the beginning of January 1979 the Pioneer Venus orbiter began recording low-frequency impulses of electromagnetic radiation at periastron which Taylor *et al.* also identified with the lightning radiation⁸. So, these observations appear to confirm our interpretation. The high lightning rate on Venus apparently makes it possible to explain its glowing on the nightside. Calculation shows that for the phase angle of 90° (when the half of the planet is illuminated) the light flow from the nightside can be 10^{-4} of the reflected sunlight if the thunderstorm activity covers 4–38% of the planetary surface depending on the efficiency of the transformation of lightning energy into light⁹.

Intensive and frequent discharges of lightning (up to 50 s^{-1} in one active region) occur in the Venus cloud layer. The origin of some gaseous components of the atmosphere can be associated with this lightning. During the Venera 11 and Venera 12 investigations the thunderstorms were of a local nature. The high lightning activity on Venus can explain the nightside glowing observed from the Earth. Lightning phenomena are, therefore, typical of the Earth and also of other planets with rather dense atmospheres and clouds where the conditions necessary for great charges to accumulate are realised.

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Is something wrong with the binary pulsar?

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The binary pulsar PSR1913+16 is at present the most interesting object for measuring general relativistic effects¹ such as the periastron advance, emission of gravitational waves, and gravitational redshift. It also offers unique possibilities for studying the properties of one or two neutron stars. Yet several of the observed effects could be simulated by an extended secondary, and recent optical measurements^{2,3} have suggested that such is the case, at the 97% confidence level. Here I shall argue that the optical source seen at the position of PSR1913+16 may be the pulsar sphere, that is, a region of confined extremely relativistic plasma glowing in the near IR, and that the secondary is another neutron star (as preferred by most authors). Therefore, the optical coincidence need not cause us to doubt the published general relativistic analysis of this system. I shall also comment on the binary pulsar's likely history, and on the possible presence of a second binary pulsar in Cas A.

At its present spindown rate¹ of $\dot{P} = 0.864 \times 10^{-17}$ and period $P = 0.0590 \text{ s}$, the binary pulsar loses rotational energy at a rate

$$-\dot{E} = -I\Omega\dot{\Omega} = 10^{33} I_{44.8} \text{ erg s}^{-1} \quad (1)$$

where $\Omega := 2\pi/P$. I is the moment of inertia, and

$I_{44.8} := I/10^{44.8} \text{ g cm}^2$ equals unity for the Crab pulsar⁴. Where does this power go?

In the case of the Crab, the pulsar is thought⁵ to illuminate the surrounding supernova remnant; that is, a substantial fraction of its rotational energy losses is thought to be converted into magnetobremstrahlung of the Crab nebula. More quantitatively⁴, half of the corresponding power may be pumped into the 30-Hz wave bath, and post-accelerate the expanding filaments as well as heat the lowest-energy relativistic electrons. The other half of that power is thought to be injected into the nebula in the form of highly relativistic electrons and positrons, with Lorentz factors γ in the range $3 \times 10^5 \leq \gamma \leq 10^7$, distributed according to $N_\gamma d\gamma \sim \gamma^{-2.2} d\gamma$. In the nebular electromagnetic field of strength $\leq 10^{-3}$ G, these particles lose their energy via magnetobremstrahlung whose power peaks at the frequency

$$\nu_B = eB_\perp \gamma^2 / \pi m_e c = 6 \times 10^{15} \text{ Hz } B_{-3} \gamma_6^2 \quad (2)$$

that is, in the UV.

If the Vela pulsar has the same moment of inertia as the Crab pulsar, some 10% of its spindown power escape as pulsed γ rays^{6,7}, between 0.3 and 3 GeV. Luminosities at radio frequencies⁸ and X-ray energies⁹ are about 10 times smaller than at γ -ray energies. In analogy to the Crab, more than half of the spindown power is expected to be converted into magnetobremstrahlung (= synchrotron, or synchro-Compton radiation), probably at IR frequencies. Because of the large angular extent of the source, such a flux is difficult to measure. The magnetic fields in Vela X may be of the order⁹ of $10^{-4.5 \pm 0.5}$ G, so that the observed parts of the spectrum and equation (2) imply injected Lorentz factors in the interval $10^5 \leq \gamma \leq 10^8$.

The binary pulsar has a period between those of the Crab and Vela pulsars, and an inferred surface field strength

$$B_s = (3c^3 |\dot{E}| / 2R^6 \Omega^4)^{1/2} = 1.8 \times 10^{10} \text{ G} \quad (3)$$

which is more than 10^2 times weaker. Here a standard neutron star radius $R = 10$ km has been assumed, and a perpendicular magnetic dipole field responsible for the radio pulses. The binary pulsar's spindown age, $t = P/2\dot{P} = 1.1 \times 10^8$ yr may be an overestimate of its true age; but t is certainly a measure of the pulsar's present ageing rate or lifetime. If it were a large overestimate of its true age, by a factor N , we should expect, on statistical grounds, to see N more binary pulsars in the sky.

As supernova remnants containing pulsars (not shrouded neutron stars) seem to disperse within little more than 10^4 yr, the binary pulsar will presently find itself surrounded by typical interstellar matter, with a static pressure $p_{\text{stat}} \leq 10^{-12} \text{ dyn cm}^{-2}$, and a hydrogen density $n = T_4^{-1} \text{ cm}^{-3}$. Its relativistic wind will be confined by this matter, and run into an inner shock at a distance r_i where the wind pressure $|\dot{E}|/4\pi r_i^2 c$ meets the cavity pressure p_{cav} . In a steady-state flow, p_{cav} equals the ram pressure

$$\rho v^2 / 2 \leq 10^{-9} n_0 v_{7.5}^2 \text{ dyn cm}^{-2} \quad (4)$$

of the ambient medium, where $v = 10^{7.5} \text{ cm s}^{-1}$ is the assumed relative velocity brought about by two successive supernova explosions, see equation (8), and the inner cavity radius is

$$r_i = (|\dot{E}|/4\pi c p_{\text{cav}})^{1/2} \geq 2 \times 10^{15} p_{-9}^{-1/2} \text{ cm} \quad (5)$$

The cavity must have a larger transverse than longitudinal extent for a large pressure anisotropy $p_{\text{ram}}/p_{\text{stat}} \gg 1$ of the confining surroundings. Its size is controlled by the condition that the pulsar's particle supply must leave through its outer edge at a speed v_{esc} typical for the (much heavier) ambient medium, which implies $r_0/r_i \approx (c/v_{\text{esc}})^{1/2} \leq 30$ for the outer radial scale r_0 , or

$$r_0 \leq 10^{17} p_{-9}^{-1/2} \text{ cm} \quad (6)$$

The magnetic field inside the cavity will at least reach pressure balance strength $B = 2 \times 10^{-4} p_{-9}^{-1/2} \text{ G}$. This value is even likely to be a large underestimate because the cavity boundary is strongly Rayleigh-Taylor unstable, and intruding filaments of interstellar matter will be wrapped into much stronger magnetic fields¹⁰, reminiscent of the magnetic knots in Cas A. The extremely relativistic electrons traversing the cavity have a synchrotron lifetime

$$t_{\text{syn}} \approx (m_e^5 c^9 / e^7 B^3 \nu)^{1/2} = 0.8 \times 10^3 \text{ yr } B_{-3.5}^{-3/2} \nu_{14}^{-1/2} \quad (7)$$

if radiating at the frequency ν in a field of $10^{-3.5}$ G. At a typical gyrocentre speed of $10^{7.5} \text{ cm s}^{-1}$, they therefore lose a significant fraction of their energy over a distance of several 10^{17} cm $B_{-3.5}^{-3/2} \nu_{14}^{-1/2}$. For an effective cavity radius of 10^{17} cm , particles may traverse some $3 \times 10^{17} \text{ cm}$ before they leave the vicinity of the pulsar, and are dragged along by the wake into regions of reduced pressure and much lower synchrotron brightness. Consequently, from a distance of $d = 5$ kpc the pulsar sphere is expected to look like a source of angular diameter ≤ 2.8 arc s $d_{22.2}^{-1} p_{-9}^{-1/2}$, whilst the reported 'star' has an unresolved size of ≤ 2 arc s in the deep red band³. (See also ref. 11 which excludes pulsations at the 1% level.) I propose that the reported star is in fact the glowing pulsar sphere.

This interpretation is strengthened by the fact that from the observations by Kristian *et al.*² and Crane *et al.*³ one can derive an $R - V$ spectral index $\alpha = -2.6$, ($S_\nu \sim \nu^\alpha$), very unlike that of a hot star; of course, α carries a large uncertainty. The detected flux of $1.6 \times 10^{-5} \text{ Jy}$ at $0.68 \times 10^{-4} \text{ cm}$ corresponds to an R -luminosity of $L_R = 2 \times 10^{32} \text{ erg s}^{-1} = 0.2 |\dot{E}_{\text{PSR}}|$, in agreement with the model. Note that an additional power input of $\pi r_0^2 \rho v^3 / 2 = 5 \times 10^{30} \text{ erg s}^{-1} r_{17}^2 v_{7.5}^3$ is expected from the ram pressure exerted against the interstellar medium, with voltages of the order of $2 r_0 e B_\infty v / c = 10^{12} \text{ eV } B_{-5} v_{7.5}$. This power is, however, small unless v exceeded 10^3 km s^{-1} .

The proposed identification of the binary pulsar's sphere with the starlike optical source near $(l, b) = (49.97^\circ, 2.1^\circ)$ is based on an assumed distance ≥ 5 kpc corresponding to the dispersion measure $171.64 \text{ pc cm}^{-3}$. If the pulsar's rays crossed (E. Schröder, personal communication) the Strömgren sphere of the star at $(l, b) = (49.6^\circ, 0.25^\circ)$ which has been tentatively classified as OV, the dispersion measure could be a large overestimate of distance, and the binary pulsar could be as near as 0.8 kpc. In view of the small observed variability (L. A. Fowler, personal communication) of the dispersion measure [$< 0.6 \times 10^{-4}$], however, this possibility is not very likely.

Are the binary pulsar's properties exceptional? Its low inferred surface field strength (equation (3)) has sometimes led to the conjecture¹² that it is the older of the two hypothetical neutron stars, and that it was born with a much higher magnetic field. There is, however, as yet no conclusive evidence that neutron star magnetic fields do decay on a time scale of 10^8 yr; see refs 13–15. If pulsar dipole fields did decay, they would have to do so with a rather wide range of decay times, between $\sim 10^7$ and 10^{10} yr. (But magnetic field decay may have taken place in the—probably $\sim 10^{10}$ yr old—X-ray bursters¹⁶.) But there is significant evidence that binary neutron stars are spun down by the wind of their companion star^{17,18} to periods which are in most cases too long to make them visible as pulsars. The older of the two neutron stars may hence be spinning too slowly to be detected. Why then does the younger neutron star lack a strong magnetic field?

The possible evolutionary histories of the binary pulsar have been carefully discussed elsewhere^{12,19,20}. In view of the more recent data, the most convincing configuration before the last supernova explosion in the system is a neutron star in close circular orbit around a He star. One can then calculate the presupernova parameters of the system under the idealising assumptions of: (1) an initially circular relative orbit; (2) an instantaneous and symmetrical explosion; and (3) vanishing momentum transfer to the companion during the explosion. The condition of coincident initial conditions at periastron yields¹²

$$\begin{aligned} (M_1^- - M_1)/M &= \epsilon = 0.617 \\ M_1^- &= M_1 + \epsilon(M_1 + M_2) = 3.0 M_\odot \\ a^- &= (1 - \epsilon)a = 0.747 \times 10^{11} \text{ cm} \quad (8) \\ \omega^- &= [(1 + \epsilon)/(1 - \epsilon)^3]^{1/2} \omega = 2\pi/1.44 \text{ h} \\ v_{\text{rec}} &= \epsilon(1 - \epsilon^2)^{-1/2} (M_2/M)(GM/a)^{1/2} \\ &= 1.8 \times 10^7 \text{ cm s}^{-1} \end{aligned}$$

where superscript ‘-’ denotes presupernova values, ε = numerical eccentricity, a = semi-major axis, ω = orbital angular velocity, v_{rec} = recoil velocity, and $M_1 = (1.3 \pm 0.15) M_{\odot}$, $M_2 = (1.5 \pm 0.15) M_{\odot}$ are the best estimates at present (L. A. Fowler, personal communication) for the masses, with $M := M_1 + M_2 = 2.83 M_{\odot}$. Obviously, the system could not get disrupted in the explosion because there was not enough mass in the shell. The ‘missing mass’ in the shell may have been evaporated by the closely orbiting (first) neutron star¹². Hence an exceptional property of the binary pulsar (when compared with other pulsars) is the small separation of its progenitor system, which in turn may have resulted from an exceptionally small mass ratio of the massive binary from which it descended²¹.

I shall now argue that a close presupernova orbit is likely to imply a small magnetic dipole moment of the resulting neutron star, and may hence be the only exceptional property of the binary pulsar. The argument follows Ruderman and Sutherland²² in that the neutron star inherits its magnetic moment from the convective core of its progenitor star, whose equipartition magnetic flux decreases with decreasing convective motion and hence with decreasing spin. But a presupernova binary separation of one solar radius gives rise to such a strong tidal couple that near-synchronous rotation is enforced within²³

$$\tau_1^{\text{synch}} \approx \frac{\alpha}{K} \left(\frac{M_1}{M_2} \right)^3 \left(\frac{a}{R_1} \right)^9 \frac{1}{\omega} \approx 10^{-2} \text{yr} \left(\frac{a}{R_1} \right)^9, \quad (9)$$

where $\alpha/K \approx 8$, and R_1 is the radius of the helium star (through atmosphere of which the neutron star orbits). A spin period of the core of more than 1 h is still short enough to give rise to a fast pulsar, but probably not fast enough to drive a strong dynamo²².

Why may the older of the two neutron stars have a higher mass? It is tempting to speculate that it has accreted some $0.1 M_{\odot}$ throughout several Myr from the atmosphere of its companion.

The binary pulsar, therefore, (still) allows us to think of neutron stars as objects with standard properties produced by ideal supernovae. All that may be special about it is the small separation of its progenitors, which prevents disruption, implies a small magnetic moment, a low luminosity, and high space velocity (imparted both by the first and second supernova explosion).

An even larger space velocity must have been imparted to the (light) supernova shell blown off when the binary pulsar was born (due to momentum conservation). As I have argued previously¹⁰, a light supernova shell [$< 3 M_{\odot}$] with a high space velocity [of $(165 \pm 15) \text{km s}^{-1}$] is Cas A. Cas A is exceptional for its radio brightness: Tammann²⁴ estimates that at most 2% of all supernova remnants are similar to it. Its recoil partner should presently be located at (23 h 21 min $(12 \pm 0.5) \text{s}$, $58^\circ 32' (17 \pm 1)''$). As no (strong) pulsar nor bright star has been found in this region, a (weak) binary pulsar is a likely candidate. It could, perhaps, be the third²⁵ binary system in which emission of gravitational waves is measurable.

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Magnetic energy conversion, magnetospheric substorms and solar flares

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An explosive conversion of magnetic energy has long been suggested as the cause of various electrodynamic processes in astrophysical conditions, such as auroral magnetospheric substorms, solar flares, stellar flares and some galactic scale processes^{1–6}. It is generally believed that the magnetic energy is stored in a certain region before the onset of those phenomena and that the conversion takes place spontaneously or by triggering. Here it is suggested that the magnetospheric substorm is a direct consequence of increasing power generated by the solar wind–magnetosphere dynamo, rather than of a hypothetical sudden conversion of the stored magnetic energy.

The magnetospheric substorm has been thought to be the manifestation of a sudden conversion of the magnetic energy stored in the magnetotail before substorm onset. It has been believed that solar flares arise from an explosive conversion of the magnetic energy stored in the coronal level above a large sunspot group. Among these and stellar situations, the magnetosphere is the only region which can be ‘diagnosed’ systematically by *in situ* measurements of plasma quantities, as well as of electric and magnetic fields, providing the grounds to test the hypothesis of a sudden magnetic energy conversion. The solar wind (a magnetised plasma flow) and the magnetosphere (a magnetised celestial body) constitute a dynamo. The dynamo-induced current flows in various parts of the magnetosphere, including the magnetotail and the ionosphere. Therefore, by comparing time variations of the input energy flow into the magnetosphere, the amount of the magnetic energy in the magnetotail and the energy consumed by the magnetosphere, one should be able to identify what processes are involved in magnetospheric substorms.

We have recently found a particular solar wind quantity ε which correlates well with the rate of the total energy \dot{U} consumed by the magnetosphere⁷. It is given by:

$$\varepsilon = VB^2 \sin^2 \left(\frac{\theta}{2} \right) l_0^2 \quad (1)$$

where V (cm s^{-1}) is the solar wind speed; B (G) is the magnitude of the interplanetary magnetic field; θ is the polar angle of the IMF projected onto a plane perpendicular to the Sun–Earth line; l_0 is constant ($= 7R_E$); and \dot{U} is the sum of the rate of the Joule heat production in the ionosphere \dot{U}_i , the rate of the ring current particle in injection \dot{U}_R , and the rate of auroral particle injection \dot{U}_A .

Furthermore, it has been shown⁸ that ε is equal to the power P generated by the solar wind–magnetosphere dynamo, which is given by⁹

$$P = \frac{1}{4\pi} (B_{\text{tan}} B_{\text{norm}}) VS \quad (2)$$

where B_{tan} and B_{norm} denote the tangential and normal components of the magnetic field at the magnetopause and S denotes the surface area of the magnetotail.

It is thus of great interest to examine the relationship among the solar wind–magnetosphere energy coupling function (ε), the

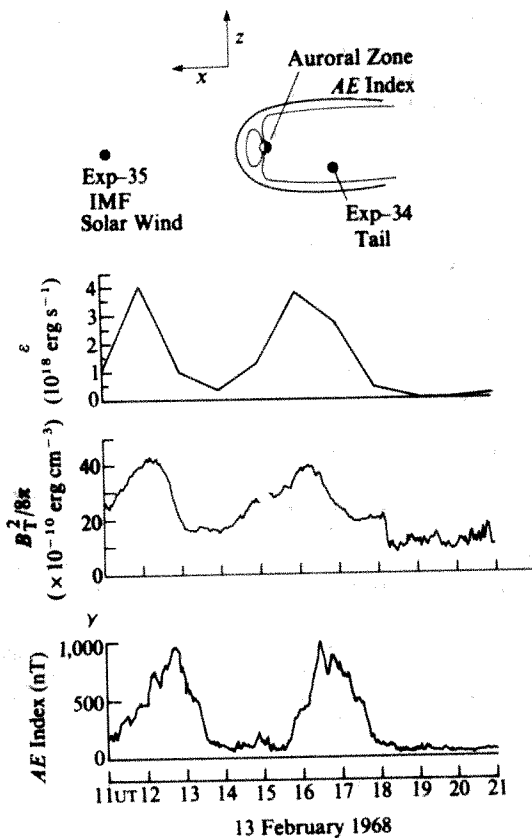


Fig. 1 Comparison of the solar wind-magnetosphere energy coupling function ϵ , the magnetic energy density $B_T^2/8\pi$ in the high latitude lobe in the magnetotail, and the magnetospheric substorm index AE . These data were obtained at Explorer 35 and 34 satellites and auroral zone stations, respectively, and their locations with respect to the magnetosphere are shown in the insert.

magnetic energy density ($B_T^2/8\pi$) in the magnetotail, where B_T denotes the magnetotail field in the high latitude lobe, and the substorm index $AE(nT) \approx \bar{U}_1/10^{15} \text{ erg s}^{-1}$ (ref. 10). Figure 1 shows the relationship between these three quantities monitored by the Explorer 34, 35 satellites and auroral zone magnetic observatories, respectively. This particular magnetotail event was chosen because it has been well documented¹¹. For the relative location of the satellites with respect to the magnetosphere, see the insert in Fig. 1.

Figure 1 shows that the three quantities, ϵ , $B_T^2/8\pi$ and AE vary roughly in harmony. Note particularly the occurrence of two substorms which are clearly indicated by an increase of the AE index; the corresponding auroral activity was well recorded in Alaska. If one assumes that the magnetospheric substorm is produced by an explosive conversion of the magnetic energy stored in the magnetotail before substorm onset, $B_T^2/8\pi$ should begin to decrease rapidly at $T=0$, namely at ~ 11.00 UT and ~ 15.30 UT, respectively. On the contrary, $B_T^2/8\pi$ increased with ϵ and the AE index at least during the main epoch (11.00–12.10 UT and 15.30–16.20 UT) of the expansive phase.

An important implication of this result is that the development of magnetospheric substorms is a direct consequence of increasing ϵ and the solar wind-magnetosphere dynamo power P . As the power is increased, both the magnetotail (solenoidal) current and the auroral electrojet are enhanced, as can be seen in the corresponding increase of $B_T^2/8\pi$ and AE . Note, however, that we assume here that the total volume of the magnetotail is not reduced appreciably. It has already been shown that ϵ and the AE index are related statistically by $AE(nT) \approx \epsilon/(10^{16} \text{ erg s}^{-1})$ for $\epsilon < 10^{19} \text{ erg s}^{-1}$ (ref. 10) although in this particular case, $AE(nT) \approx 2\epsilon/(10^{16} \text{ erg s}^{-1})$. Auroral activity manifests typical features of the substorm when the accompanying magnetic variations exceed about 100 nT (ref. 12); so that substorm activity is associated with $\epsilon \geq 10^{18} \text{ erg s}^{-1}$.

Note that although hourly values are used in computing ϵ , there seems to be an appreciable time delay between $\epsilon(t)$ and $AE(t)$. Such a delay can easily be expected for the magnetospheric circuit, as its inductance is known to be of the order of ~ 100 –500 H. Since the total ionospheric resistance is of the order of 0.1 Ω , the time constant of the system is of the order of ~ 17 –85 min. Thus, the magnetosphere cannot respond instantly and fully to changes of ϵ . Any storing of energy should occur during the period when the dynamo power is increasing, not before substorm onset.

On the basis of Fig. 1 and similar events, there is little doubt that the magnetospheric substorm develops when the solar wind-magnetosphere dynamo is being driven harder than during a quiet period. The magnetospheric substorm is not simply a process by which the total magnetic energy is stored before its onset and then is explosively consumed. Further, it begins to subside as ϵ decreases, not because stored energy is consumed. Therefore, this clarifies why the magnetic energy conversion subsides at the end of substorms (as a large amount of magnetic energy is still available then or even during a quiet time).

The magnetospheric substorm may be said to be a direct consequence of increasing power generated by the solar wind-magnetosphere dynamo above $\epsilon > 10^{18} \text{ erg s}^{-1}$. Part of the increased current is channelled to the ionosphere along magnetic field lines. There are at least two important consequences of the increased field-aligned currents. The first is that the field-aligned currents develop a particular potential structure (called the V-shaped potential)¹³. As a result, the current-carrying electrons are accelerated downward and produce the aurora as they collide with polar upper atmosphere particles. As auroral electrons are accelerated to a few kilovolts, the auroral arc will suddenly brighten. An increased ionisation and conductivity might have a positive nonlinear effect¹⁴. The second consequence of the increased field-aligned currents is the generation of the auroral electrojet; a large increase of the Joule heat production (responding to a large increase of the power generation) can take place.

It may perhaps be premature to generalise this finding to other phenomena which have been suspected to be caused by a sudden magnetic energy conversion. On the other hand, the magnetosphere is the only region in which the hypothesis of an explosive magnetic energy conversion can be tested¹⁵. In this respect, solar flares have also been believed to be due to such an energy conversion process. Indeed, there is a close phenomenological similarity between magnetospheric substorms and solar flares; in fact, flare ribbons may be identified as solar auroras¹⁶. Although details are given elsewhere¹⁶, note that for a vertical wind of speed $V = 1 \text{ km s}^{-1}$ across horizontal magnetic field lines of $B = 500 \text{ G}$ in an area S of $10^5 \text{ km} \times 10^5 \text{ km}$, the dynamo power is given by $P = (B^2/8\pi) \times S \times V \approx 10^{29} \text{ erg s}^{-1}$. Therefore, as in the case of auroral substorm, in spite of its sudden onset, a solar flare could be a direct result of a photospheric dynamo process. In fact, it has been shown that a solar flare tends to occur in the vicinity of a newly emerging magnetic flux¹⁷, so that this emerging flux is likely to be an indication of the presence of such a dynamo process.

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The effect of compressible flow on anti-dynamo theorems

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The 'anti-dynamo' theorems (ADTs) impose restrictions on the symmetry properties of the velocity and magnetic fields associated with sustained dynamo action in an electrically conducting body of fluid, such as the Earth's core. However, several of these ADTs depend on the assumption that the fluid flow is incompressible, and we show here that their validity is queried when compressible flow is considered. In particular, non-stationary axisymmetric dynamos may no longer be prohibited by Cowling's theorem. The high degree of axisymmetry of Saturn's magnetic dipole, revealed by the recent Pioneer 11 flyby, may be related to the planet's large compressibility.

The first and best-known of the ADTs is Cowling's¹ (extended by Backus and Chandrasekhar²), which proves the impossibility of supporting a stationary axisymmetric magnetic field **B** by axisymmetric fluid motions. The essential point in Cowling's argument is that motional induction cannot maintain **B** in the vicinity of the 'neutral' curve **B** = 0, so the latter region is a sink of magnetic energy¹. If the requirement of stationarity is relaxed, this decay may be offset by diffusion into this region of field produced elsewhere in the conducting fluid body, and the proof of the ADT apparently fails. However, Cowling's theorem was later^{3,4} generalised, using a different proof based on global properties of the field, to forbid even non-stationary axisymmetric dynamos.

There seems to be a widespread impression that Lortz⁵ has shown that even the requirement of axisymmetry in the velocity field **v** can be abandoned without weakening Cowling's theorem, but Lortz's argument rests subtly on an assumption about axisymmetry of the electric field which in turn forces **v** to be axisymmetric.

Cowling's theorem has played an important part in the development of dynamo theory. It contributed to the scepticism with which several eminent physicists greeted Elsasser's pioneering studies in the 1940s and early 1950s. The widely felt suspicion that a more general and thoroughly prohibitive ADT might exist was not totally dispelled until 'existence proofs' were found independently by Herzenberg and by Backus in 1958. The necessity of avoiding the consequences of Cowling's theorem also decisively guided the Bullard-Gellman-Lilley-Gubbins and Braginskii approaches to a plausible terrestrial dynamo model.

Elsasser⁶ argued convincingly, and rigorous proofs were provided by Bullard and Gellman⁷ and by Backus⁸, that a toroidal **v** could not maintain dynamo action. This ADT has generally been interpreted as requiring **v** to have a non-zero radial component in a working dynamo. Analogous to this constraint is the impossibility of dynamo action when **v** lacks a component in one cartesian direction⁹. Cowling³ recognised that the toroidal **v**, two-dimensional, and non-stationary axisymmetric ADTs are but special cases of a single class of theorems whose proofs use global properties of **B** and **v**. Other ADTs have been conjectured¹⁰, but apparently the only other for which a rigorous proof exists is that prohibiting a stationary dynamo in a sphere when **v** is purely radial¹¹.

An almost universal assumption in dynamo theory is that the fluid flow can be treated as incompressible—that **v** is solenoidal, $\nabla \cdot \mathbf{v} = 0$. An exception to this is the paper by Namikawa and Matsushita¹¹, who remark that "a curl-free velocity field may be important for the dynamo maintenance of the magnetic field in the Earth's core". Also Krause and Roberts¹² find that, when

the electrical conductivity is low enough, finite compressibility can assist dynamo action in mirror-symmetric turbulence, but not enough to actually bring about regeneration.

As Smylie and Rochester¹³ have shown, the assumption of solenoidal flow is not permissible for large-scale radial motions in the stratified liquid core of the Earth. The relative importance of compression by transport through the pre-existing hydrostatic pressure field is measured by the dimensionless number $C = gL/c^2$, where g and c are representative values for gravity and compressional wave speed in the liquid core, and L is the radial length scale of the motion. Only for $C \ll 1$ can the flow be treated as solenoidal. For the Earth's core, $C \sim 0.1$ when $L \sim 10^6$ m. The neglect of compressibility may therefore be of questionable validity for dynamo mechanisms which do not depend on small-scale turbulence in the core.

Among the most interesting consequences of taking compressibility into account are those for the ADTs which rest on 'global' proofs. Cowling's original 'neutral-curve' argument¹ is not dependent on any assumption of solenoidal flow, so his theorem forbidding a strictly stationary axisymmetric dynamo is unaffected. The same is true of the radial-**v** ADT discovered by Namikawa and Matsushita¹¹. But the proofs^{3,4} of the three other ADTs, using global properties of the fields, fail when a lamellar part $\nabla \mathcal{L}$ is added to **v**. Separate clear standard proofs for each of these ADTs have been collected recently by Moffatt¹⁴.

The 'uncurled' hydromagnetic induction equation for axisymmetric **B** and **v** leads to the equation

$$\frac{d}{dt} \int_{V_\infty} \frac{\chi^2}{2\lambda} dV + \int_{V_\infty} (\nabla \chi)^2 dV = \int_{V_\infty} \frac{\chi^2}{2\lambda} \nabla^2 \mathcal{L} dV \quad (1)$$

where λ is the magnetic diffusivity, V_∞ is all space, and $\chi = -r \sin \theta (\partial P / \partial \theta)$ is the flux function associated with the poloidal part of **B**, $\mathbf{B} = \nabla \chi \times (\nabla \times \mathbf{r} P)$. Here r and θ are the usual spherical polar coordinates. If the RHS of equation (1) vanishes, we have Moffatt's equation (6.24), from which it is clear that the poloidal magnetic field must ultimately decay. But this conclusion is no longer assured for flows such that $\nabla^2 \mathcal{L}$ is non-negative in some finite region of space. A similar result is obtained for the toroidal part of **B** by the appropriate modification of Moffatt's equation (6.27). If U is a representative value of the radial component of the lamellar part of **v**, then $\nabla \cdot \mathbf{v} \sim UC/L$, and equation (1) indicates that the proof of this ADT is lost if $UC/L > 2\lambda/L^2$, that is if $CR_m > 2$, where $R_m = UL/\lambda$ is the magnetic Reynolds number. This requirement does not seem too difficult to meet in the Earth's core, and should be even easier to satisfy in an even more compressible planet such as Saturn. The possibility that non-stationary axisymmetric dynamos can be sustained by lamellar flow deserves investigation.

By definition a toroidal **v** is solenoidal and has no radial component. If the first of these conditions is relaxed as above, but with $\partial \mathcal{L} / \partial r = 0$, Moffatt's equation (6.41) is replaced by the equation

$$\frac{d}{dt} \int_V Q^2 dV + 2\lambda \int_V (\nabla Q)^2 dV = - \int_V Q^2 \nabla^2 \mathcal{L} dV \quad (2)$$

where $Q = \mathbf{r} \cdot \mathbf{B}$, and V is the volume of the conducting fluid (in which λ is supposed uniform). When its RHS vanishes, equation (2), together with Moffatt's equation (6.46) (which is similarly affected by lamellar flow), yields the ADT for toroidal **v**. But clearly the usual interpretation (that a radial component of **v** is required for dynamo action) may be voided if sufficiently compressible flow is allowed. However, because of the large non-radial compression required to violate it, the proof of this ADT for non-radial **v** remains valid in the Earth's core, at least. The prohibition against dynamos driven by plane two-dimensional motion⁹ may be similarly withdrawn in principle by permitting compressible flow.

We conclude that the usual proofs of several ADTs are nullified by abandoning the requirement of strictly solenoidal flow. We do not yet know if this means that the theorems themselves are invalid for compressible flow—that alternative

proofs independent of the incompressibility assumption cannot (or that counter examples can) be found. We do suggest that allowing for the physically real compressibility of electrically conducting fluids in planets may considerably relax (or remove) the constraint imposed by one of the ADTs, and permit the construction of non-stationary axisymmetric dynamos. For evidence that this result is not of purely academic interest we cite: (1) the likelihood, based on the known history of the Earth's magnetic field, that physical dynamos are essentially nonstationary on a wide range of time scales; (2) the recent discovery by Pioneer 11, that Saturn's magnetic dipole is highly axisymmetric¹⁵.

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Anomalous high uranium contents in the sediment under Galapagos hydrothermal mounds

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The geochemical behaviour of uranium and thorium in metalliferous sediments and hydrothermal deposits has been widely studied and the main results have been summarised by Boström and Rydell¹. These isotopes may be used to clarify how the metal-rich solutions are introduced into sediment cover and seawater. Using radiochemistry followed by α spectrometry, we have measured uranium concentrations as high as several hundred p.p.m., which must clearly be associated with ocean ridge thermal activity, in sediments interbedded between the basaltic basement and the green hydrothermal mud at DSDP Site 424. These high uranium concentrations indicate the path followed by the hydrothermal fluid which, debouching at the sediment–water interface, formed the green mud.

Very high uranium concentrations have been reported in East Pacific Rise (EPR) metalliferous sediments by Boström and Rydell¹ who found, on a carbonate-free basis, average value of 38 p.p.m. (maximum 62 p.p.m.). In the metalliferous sediments of Red Sea Brines, a maximum value of 31.3 p.p.m. has been found². The highest value presently reported for uranium concentration is 150 p.p.m. (CaCO₃-free basis) for a level in core GS-7202-35 (14°47' S, 113°30' W)³. However, in metalliferous sediments near the basaltic basement at DSDP sites 37, 38 and 39, only a weak enrichment in uranium was found⁴. The high concentrations are generally attributed to the scavenging of seawater uranium by the precipitation of iron hydroxides when hydrothermal solutions debouch in the seawater as the ²³⁴U/²³⁸U ratio in these deposits is close to the isotopic ratio of seawater. When higher isotopic ratios are found, they are considered to be due to hydrothermal leaching from basalt without subsequent important mixing with seawater uranium; the uranium content is generally relatively low⁵.

During DSDP Leg 54, four holes have been drilled at Site 424, an area of mounds previously described^{6–9} and of presumably hydrothermal origin. A relatively detailed description of the drill-holes (424, 424 A, B and C) drilled about 22 km south of the spreading axis (00°35' N, 86°07' W) is given by Hékinian *et al.*¹⁰ and Natland *et al.*¹¹. Some lithological data are available^{10,11}: the mean sedimentation rate in the area, calculated from Site 425 is 4.5 cm kyr⁻¹, if a constant and regular sedimentation rate is accepted for the 83-m thick sediment blanket covering a 1.8 Myr-old basement.

The schematic lithological sequence in holes 424, 424 A and B is, starting at the basaltic basement:

(1) a layer of pelagic sediment (referred to here as the basal sediment), essentially Foraminifera and nannocalcareous ooze, with thicknesses of 18.5, 13 and 13.5 m at each hole respectively,

(2) a relatively thick (15 m) layer of hydrothermal deposit formed with a green clay-rich material. This clay is essentially formed with nontronite,

(3) capping the whole: in holes 424 and 424 A drilled directly on the mounds a Mn oxide-rich layer is found, while in hole 424 B, between the mounds, the iron rich hydrothermal deposit is capped with calcareous sediments.

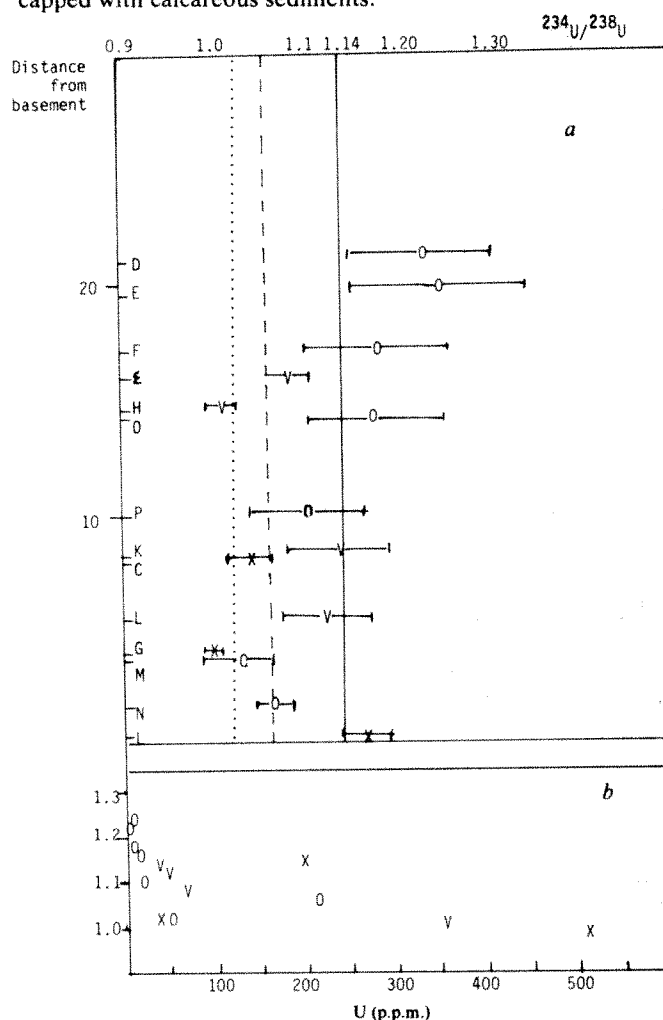


Fig. 1 a, ²³⁴U/²³⁸U activity ratio variations in the three drilled holes. Depths in the holes (m) have been arbitrarily calculated from the basaltic basement and are approximate values. Letters represent the laboratory number of the samples (see Table 1). O, values found in hole 424; V, values found in hole 424 A; X, values found in hole 424 B. Bars represent uncertainties calculated as one standard deviation (1 σ) derived from counting statistics. Solid line indicates present seawater ratio (1.14); dashed line indicates the ratio for a seawater uranium deposited 300,000 yr ago (1.060); dotted line indicates the ratio for seawater uranium deposited 600,000 yr ago (1.026). b, ²³⁴U/²³⁸U activity ratio versus uranium concentrations (symbols as in a).

Table 1 Uranium and thorium concentrations, activity ratios and percentage CaCO_3

Hole	Lab. no.	DSDP no.	^{238}U (p.p.m.)	^{232}Th (p.p.m.)	$^{234}\text{U}/^{238}\text{U}^*$	$^{234}\text{U}/^{238}\text{U}^{\dagger}$	CaCO_3 (%)
424	D	2.5. 80. 81	3.62 ± 0.28	2.33 ± 0.32	1.233 ± 0.080	1.539	58
	E	2.6. 98. 99	5.77 ± 0.54	3.38 ± 0.42	1.250 ± 0.10	1.578	76
	F	3.2. 71. 72	6.13 ± 0.46	3.97 ± 0.48	1.180 ± 0.075	1.416	69
	O	3.3.113.114	10.78 ± 0.61	5.17 ± 0.68	1.177 ± 0.075	1.409	82
	P	3.6. 85. 86	14.94 ± 0.75	4.23 ± 0.65	1.106 ± 0.062	1.245	83
	M	4.4. 90. 91	46 ± 2	7.5 ± 1.2	1.028 ± 0.042	1.065	89
	N	4.5.130.131	207 ± 7	9.0 ± 1.5	1.066 ± 0.021	1.152	89
	ϵ	2.2.125.127	61.3 ± 1.6	6.5 ± 0.6	1.089 ± 0.021	1.208	88
424 A	H	2.3.109.110	344 ± 17	11.0 ± 1.4	1.010 ± 0.016	1.023	95
	K	3.1.139.140	33.0 ± 1.7	8 ± 1	1.135 ± 0.057	1.312	92
	L	3.3.101.102	43 ± 2	10.9 ± 1.7	1.121 ± 0.051	1.28	94
424 B	C	3.5. 20. 21	35.6 ± 1.9	3.7 ± 1.1	1.037 ± 0.024	1.086	79
	G	4.1. 95. 96	510 ± 27	5 ± 1	0.989 ± 0.07		88
	I	4.3. 80. 81	196 ± 9	8 ± 2	1.160 ± 0.029	1.37	93

* Activity ratio.

 \dagger Corrected for a 300,000 yr decrease of ^{234}U excess (see text).The uncertainties are one standard deviation (1σ) derived from the counting statistics.

Natland *et al.*¹¹, using the age of the basement (about 0.6 Myr), the thickness of the basal sediment (about 15 m) and a sedimentation rate of about 5 cm kyr^{-1} , arrive at an age of 300,000 yr for the hydrothermal event. This conclusion is corroborated by the finding of two acoustic reflectors⁷ which can be attributed to this anomalous layer, the lowest one pinching on the crust, about 12 km from the Galápagos rift, on a crust about 300,000 yr old¹¹.

From the previous preliminary studies^{10,11} the two hydrothermal formations, green mud and manganese oxides, must have been deposited by two different events, the first one 300,000 yr ago, during which reducing conditions prevailed, the second one, recent and perhaps always active, in oxidising conditions.

We consider here the detailed study of the uranium and thorium content, and of the $^{234}\text{U}/^{238}\text{U}$ activity ratio in the basal sediment, deposited between 600,000 and 300,000 yr ago. The study of the distribution of these nuclides and of possible disequilibrium in the series in the hydrothermal mud, the manganese deposit and the upper sediment layer will be described elsewhere¹².

Uranium and thorium in the basal sediment were measured by α spectrometry, using either a solid state detector or a ionisation chamber, associated with a multichannel pulse height analyser. Uranium and thorium were separated and purified by co-precipitation, solvent extraction, ion exchange and final extraction with 1-(2'-thenoyl)-3,3,3-trifluoroacetone, followed by evaporation of the solution on stainless steel plates. As a spike, we used a ^{228}Th solution in secular equilibrium with ^{232}U . No measurements were made without the spike to verify that the equilibrium between natural ^{228}Th and ^{232}Th was achieved, because we only had about 1 g of sediment for each level and as ^{232}Th concentrations are so low that even if a disequilibrium were to exist, this would not greatly affect the results.

These sediments being very rich in Foraminifera and nannocalcareous ooze, which are low in uranium (0.1–0.13 p.p.m.)¹³, then dilute the authigenic or detrital uranium, the uranium and thorium concentrations are given in Table 1 on a dry and carbonate-free basis. Table 1 also shows the CaCO_3 content, the $^{234}\text{U}/^{238}\text{U}$ activity ratios, and the $^{234}\text{U}/^{238}\text{U}$ activity ratios corrected for a possible decrease during 300,000 yr.

A striking enrichment in uranium is seen, amounting to 500 p.p.m. in hole 424 B, core 4, section 1, 95–96 cm, but also affecting the basal sediment of the two other holes. In contrast, the green hydrothermal mud has a very low uranium content (0.2–0.9 p.p.m.), as does the recent sediment in hole 424 B (0.9–5 p.p.m., CaCO_3 free)¹².

In contrast ^{232}Th is depleted relative to normal pelagic clays (13 p.p.m. (ref. 14)), which may indicate that the non-carbonate

fraction contains very few detrital clays. Nevertheless, ^{232}Th concentrations are higher than in the green hydrothermal mud (about 1 p.p.m. (ref. 12)).

The first important point is that, in contrast with other metaliferous deposits, the uranium enrichment is not found in the hydrothermal deposit, but in the sediment below. Two hypotheses may explain this high uranium concentration in the sediment.

First, the uranium was concentrated from seawater in a reducing environment before the hydrothermal event which gave the green mud. In fact, in normal oxygenated marine environment uranium stays in solution, but high authigenic concentrations are encountered in shallow anaerobic sediments; concentrations amounting to 40 p.p.m. have been found in the organic-rich sediments of the Norwegian fjords¹⁵. Concentrations of the same order of magnitude associated with high molybdenum concentrations have been measured in a sediment core by Turekian and Bertine¹⁶ who interpret them "as reflecting in part the formation of local ephemeral basins along the ridge, and in part the result of the high supply of organic material". Bonatti *et al.*¹⁷ in core P 6702-59 ($20^\circ 45' \text{N}$, $85^\circ 20' \text{W}$) have found an increase of uranium content with depth that they attributed to a change in redox potential and more recently, Mangini and Dominik¹⁸ have reported high values of uranium for eastern Mediterranean sapropels.

If this origin is retained for our samples, the seawater uranium was concentrated between 600,000 and 300,000 yr ago, period during which the basal sediment accumulated. If so, according to the $2.48 \cdot 10^5$ yr half life of ^{234}U , and with the assumption of closed system for uranium, the original $^{234}\text{U}/^{238}\text{U}$ activity ratio of seawater would have decreased from 1.14 (ref. 19) to 1.060 for uranium deposited 300,000 yr ago, and to 1.026 for the one deposited 600,000 yr ago.

Figure 1a shows the values of the measured ratios. Only three present a $^{234}\text{U}/^{238}\text{U}$ activity ratio compatible with the 600,000 yr value, and two with the 300,000 yr value. All the others are higher and five of them are even higher than the present seawater value.

However, this hypothesis would not explain why low $^{234}\text{U}/^{238}\text{U}$ ratios are associated with the highest uranium content (Fig. 1b). As Ku²⁰ has already shown, the postdepositional mobility of ^{234}U means that the hypothesis of closed system for uranium in sedimentary material is no more valuable. In fact, Mangini and Dominik¹⁸ have also found, in a 28-cm thick sapropel layer, the same inverse relation between $^{234}\text{U}/^{238}\text{U}$ ratio and uranium concentration, but, in this case, associated with a strong concentration gradient. They explain the variation of $^{234}\text{U}/^{238}\text{U}$ ratio by a half closed system, in which ^{234}U excess from seawater is in a closed system, while ^{234}U *in situ* produced

by radioactive decay is mobile. This mobile fraction is able to diffuse along the concentration slope, leading to an excess of ^{234}U in the low uranium material. As Kolodny and Kaplan²¹ have shown, in phosphorite the mobility of ^{234}U is due to the differential oxidation state of ^{234}U , $^{234}\text{U}/^{238}\text{U}$ being <1 in the U(IV) fraction and >1 in the U(VI) fraction.

In the case of Mediterranean sapropels, formed during anoxic periods, the uranium certainly originates in seawater. In hole 424 sediments, the presence of benthic Foraminifera (*Cassidulina laevigata*) indicates the presence of oxygenated waters at the sediment-water interface during deposition, so that the origin of uranium cannot be seawater.

Second, the concentration of uranium is of hydrothermal origin, its precipitation being due to the reducing conditions of the hydrothermal event. This is corroborated by the fact that the hydrothermal fluid was largely reducing as it has

given reduced iron deposits, even debouching in oxygenated seawater.

Hekinian *et al.*¹⁰ proposed two models for the mode of emplacement of these hydrothermal deposits. In the first model, there is a diffuse interaction with the sediment of an hydrothermal fluid from a deep-seated source. In the second model, the mounds are the narrow vents whose fluids do not interact with the sediment. From the uranium data, we conclude that the hydrothermal fluid has percolated through the sediment. Because of the steep gradient of uranium concentration, and the quantity of uranium mobilised in such a phenomenon, we suggest that the vents are not far from the mounds.

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Possible late Quaternary pingo remnants in central London

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Active pingos are ice-cored mounds formed within permafrost¹. Actual and suspected remnants of such features have been reported widely¹⁻⁷ from former periglacial and glacial areas of the British Isles (Fig. 1)⁸⁻¹⁰. They are characteristically located in slope-foot situations with associated present-day springs or poor drainage⁴⁻⁶. Most of the observed features lie between the latitudes of The Wash and River Thames and are generally thought to have originated as open-system pingos¹¹ in the late Devensian. No pre-Devensian pingos have hitherto been recognised. Here I suggest that many of the anomalous, drift-filled, closed depressions in the surface of the rockhead (usually the London Clay) beneath some of the central London terraces, recently reviewed by Berry¹², are the partly eroded remains of former open-system pingos, dating both from the Devensian and from earlier cold stages of the Pleistocene.

These drift-filled depressions have been revealed by engineering works in central London from the late nineteenth century onwards¹². In their upper parts they seem to be of smooth contour and shaped like a wide funnel, oval in plan. In plan, the widths across their minor axes lie between about 20 and 150 m: the ratio of these to the dimensions of the major axes range generally from ~0.4 to 0.9. Their lower parts are less regular, narrower and steeper-sided (Fig. 2), in one instance featuring a buried vertical cliff of London Clay more than 12 m high. At least five of the reported depressions completely penetrate the London Clay. In these cases, the underlying stratum (generally the Woolwich and Reading Beds) has risen diapiirically into the lower parts of the depression, typically by 6-12 m. In one of the several examples where the London Clay has apparently not been penetrated, a vertical tongue of this material at least 4 m high has diapirised upwards into the base of the drift-filled depression. The maximum confirmed depth of the depressions below the general trend of the bench surface is ~25 m.

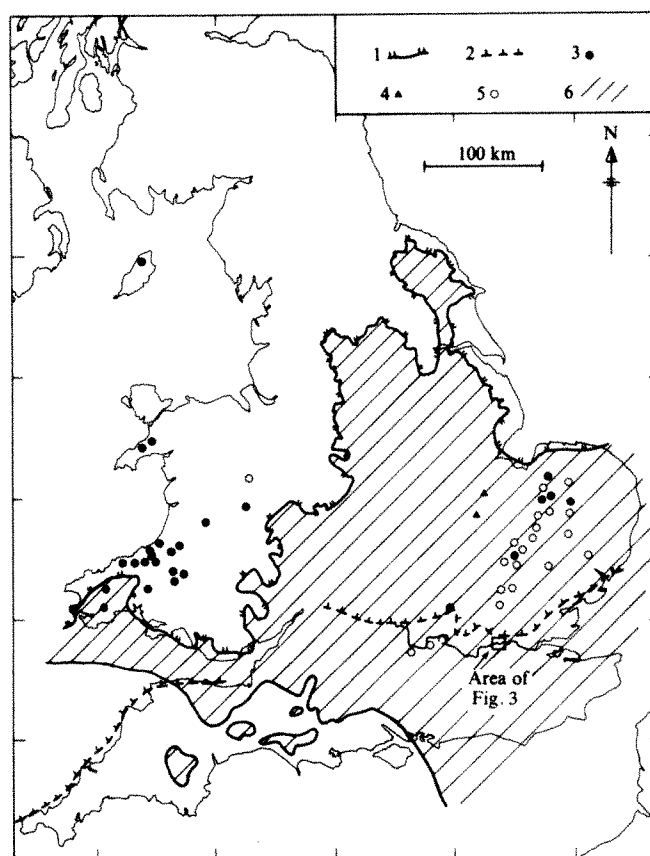


Fig. 1 Interim distribution of actual and possible pingo remnants in southern Britain, in relation to former maximum ice limits. (Acknowledgements to Committee for Aerial Photography, University of Cambridge, A. Taylor and J. Rose.) 1, Maximum southern extent of Devensian ice⁸; 2, maximum southern extent of pre-Devensian ice⁹; 3, localities of probable open-system pingo remnants in relief^{1-3,5,6}; 4, localities of possible closed-system pingos⁷; 5, localities where soil- or crop-marks indicate possible remnants of pingos or other ground ice features; 6, inferred distribution of extensive permafrost in the Devensian¹⁰.

It has been variously proposed that these features were formed by subsidence into solution cavities in the underlying Chalk¹³ (now generally discounted¹²), by some type of periglacial action^{12,14}, or by fluvial scour^{12,15}. The latter hypothesis has recently been reinforced by evidence for strong fluvial erosion during the late Devensian in stiff cohesive material in the channel of the River Gipping, Suffolk¹⁶. We accept that the anomalous depressions found in London have probably suffered major modification through fluvial scour, but do not consider that this mechanism can explain the origin of the features.

It is suggested here that many of these depressions originated as open-system pingos in the London Clay bedrock during the Devensian or earlier cold stages of the Pleistocene, deriving their water supply from artesian sub-permafrost or intra-permafrost groundwater through an unfrozen pipe or root. With the onset of interglacial or interstadial conditions, the collapsed pingo mounds would have been readily eroded away by the swollen rivers, to leave their roots exposed to local fluvial scour. This will have tended to enlarge and streamline at least the upper parts of the pingo roots, with corresponding replacement of some of the original infill by fluvial sediments. Some of the features may have been sheltered enough, or active sufficiently late, to suffer only minor modification.

The above hypothesis is supported by:

- (1) The general distribution of pingo remnants in Britain (Fig. 1), which indicates that conditions favouring the formation of open-system pingos may well have existed in the London area during the Devensian and will certainly have obtained there during earlier cold periods of the Pleistocene.
- (2) The inference, from the relation of the features to their infill, that the anomalous depressions in the London Clay were initiated in the "later stages of (late Quaternary) cold periods¹²", when the conditions of discontinuous permafrost usually associated with open-system pingos^{17,18} would have been likely to exist.
- (3) The observation that some of the depressions (such as, 3b)¹² have apparently been infilled partly by reworked London Clay^{12,19}, which may have been derived from the collapse and sloughing of a pingo mound.

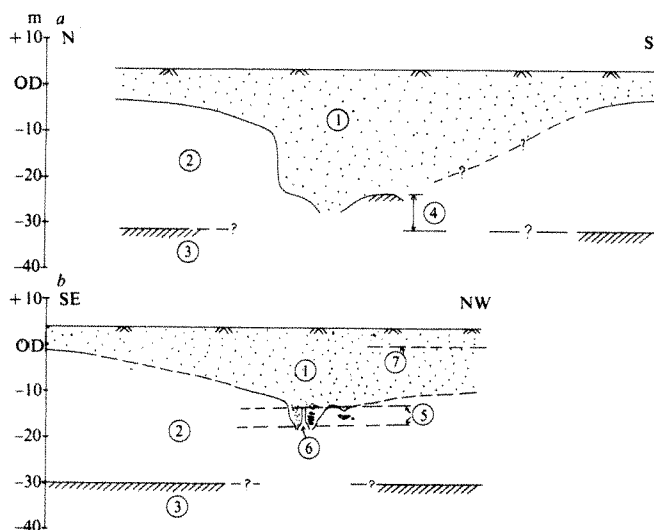


Fig. 2 Outline natural scale cross-sections of two of the anomalous depressions in central London, described by Berry¹²: a, N-S section of anomaly 1g, New Covent Garden, which penetrates the London Clay: 1, drift (chiefly sands and sandy gravels; sometimes clayey); 2, London Clay; 3, Woolwich and Reading Beds; 4, anomalous rise in level of junction of 2 and 3. b, NW-SE section of anomaly 2d, Vauxhall Grove, which appears not to penetrate the London Clay: 1-3, as for a; 5, Northbound tunnel of Victoria Line; 6, vertical tongue of London Clay; 7, general level of London Clay bench surface in the surrounding area.

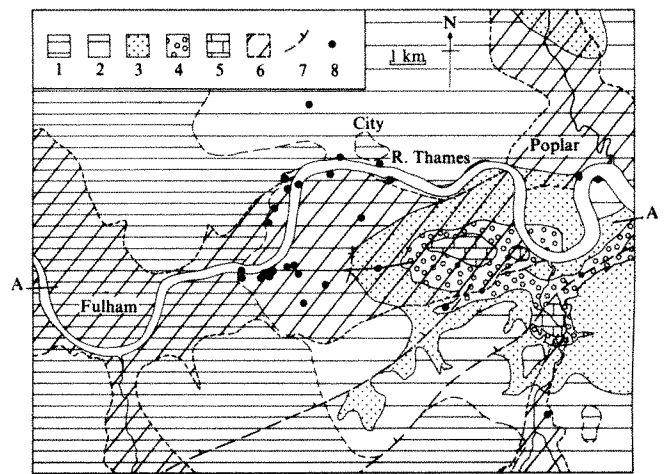


Fig. 3 Distribution of reported anomalous depressions in central London¹² in relation to the sub-crops of solid strata beneath the drift and to the estimated areas of former flowing artesian groundwater²⁰: 1, London Clay, greater than 35 m thick; 2, London Clay, less than 35 m thick; 3, Woolwich and Reading Beds; 4, Thanet Beds; 5, Upper Chalk; 6, former natural flowing artesian areas; 7, faults; 8, location of known anomalous depressions.

- (4) The distribution of the reported anomalous depressions in central London, which shows that: (i) Nearly all the depressions lie within, or close to, the former flowing artesian areas of this part of the London Basin²⁰ (Fig. 3). This suggests that groundwater conditions favouring open-system pingo formation^{11,21,22} are likely to have been present at the time when these features originated. (ii) The reported depressions occur mainly in the

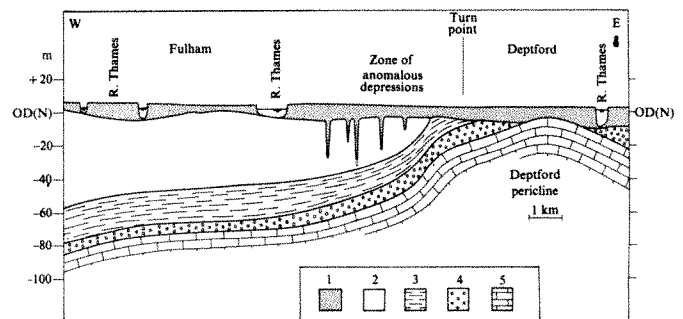


Fig. 4 Section A-A (Fig. 3) along the Thames Valley showing the relationship of the anomalous depressions to the feather-edge of the London Clay against the Deptford pericline: 1, Made ground and drift; 2, London Clay; 3, Woolwich and Reading Beds; 4, Thanet Beds; 5, Upper Chalk. Vertical exaggeration, $\times 50$.

feather edge of the London Clay stratum as it thins out against the inlier of Lower London Tertiaries and Chalk produced by the Deptford pericline (Fig. 4). In particular, they are restricted to the zone in which the present thickness of the London Clay is less than ~ 35 m (Figs 3 and 4). Bearing in mind that for open-system pingo formation a restricted supply of water is required^{11,21}, the absence of depressions from the artesian tracts where the London Clay is thicker may be the result of an inadequate upward groundwater flow. Conversely, their rarity in the artesian areas where the Lower London Tertiaries and the Chalk form the rock-head may indicate that in these localities the groundwater discharged too rapidly for pingo growth and

formed surface icings or naleds²² instead. (iii) The depressions show a propensity to cluster, generally in slope-foot or valley bottom situations; a characteristic that is entirely consistent with an open-system pingo origin^{1,17,18,21}. They are also sometimes located near stream junctions; a feature that has been taken to indicate a fluvial scour origin^{12,15}, but it is not unusual for open-system pingos to occupy such locations²³.

The deeper structures of active and fossil pingos have as yet been little explored. Nevertheless, with regard to the diapirism observed in the basal parts of the London depressions, it is noteworthy that the upward displacement of underlying strata into the base of pingo systems has been suggested in several instances²⁴⁻²⁶.

If the above proposals are valid, it will be possible to define more closely the areas of London where further anomalous depressions in the London Clay surface can be expected. Likewise, remnants of open-system pingos should be found in association with analogous artesian areas elsewhere in southern and central Britain.

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Interaction between chemoreceptive modalities of odour and irritation

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Inhaled vapours may stimulate both olfactory receptor cells and endings of the trigeminal nerve¹. The trigeminal nerve often contributes a pungent, irritating attribute to odours. Many odorants exhibit some pungency when dilute and most become pungent when concentrated². Because the trigeminal system commonly shares the chemosensory burden with olfaction, it is relevant to ask whether these anatomically distinct systems interact³⁻⁶. Two obscure psychophysical observations argue for an inhibitory influence of trigeminal over olfactory activity. Katz and Talbert observed that a vapour with both odour and pungency might lose odour at high concentrations, irritation masking odour⁷. The nineteenth century philosopher Alexander Bain, noting that concentrated carbon dioxide (carbonic acid) evokes pungency, remarked "if a current of carbonic acid accompanies an odour, the effect (odour) is arrested" (ref. 8). We have taken up Bain's forgotten observation and used carbon dioxide to endow otherwise benign concentrations of odorant with varying degrees of pungency. The experiments reported here reveal a strong mutual interaction between pungency and odour, occurring without attenuation even when irritant enters one nostril and odorant the other.

In the first experiment, eight persons judged the perceived magnitude of four concentrations of the fruity odorant *n*-amyl butyrate [0.7–12.2 parts per million (p.p.m.)], presented olfactometrically, four concentrations of carbon dioxide [10–50 parts per hundred (p.p.h.)], and all 16 combinations (mixtures) of the two. Subjects inhaled the stimuli through one nostril (flow rate: 4 l min⁻¹). They assigned numbers proportional to sensory magnitude⁹, judging a stimulus first for overall magnitude and then for either the odour component (half the trials) or the irritating component. Each subject made 240 judgments over five sessions.

In the second experiment, 10 subjects followed the same psychophysical procedure with dichorhnic 'mixtures' (4 l min⁻¹ flow rate into each nostril with equal numbers of right-left and left-right combinations of odorant and irritant). In such condi-

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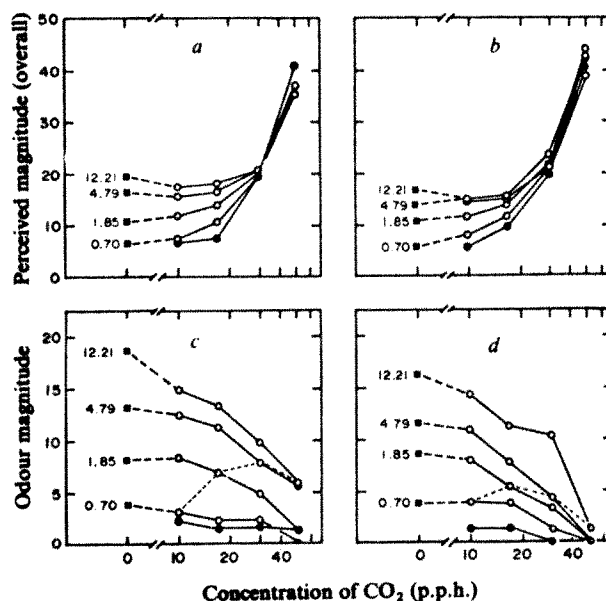


Fig. 1 a, Perceived magnitude (linear scale) plotted against concentration of carbon dioxide (logarithmic scale) for carbon dioxide presented alone (●), amyl butyrate presented alone (■) and mixtures of carbon dioxide and amyl butyrate (○). The concentration of amyl butyrate (p.p.m.) is indicated at the left in each case. Data points are medians taken across eight subjects. b, Same as a, but combinations of carbon dioxide and amyl butyrate were presented dichorhnically, that is irritant (carbon dioxide) to one nostril and odorant (amyl butyrate) to the other. Data points are medians taken across 10 subjects. c, Perceived odour component (denoted odour magnitude) of amyl butyrate alone (■), carbon dioxide (●) and physical mixtures (○). The low, but non-zero judgments for the 'odour' of the odourless irritant carbon dioxide presumably reflect imperfect perceptual resolution between odour and irritation. The non-monotonic function formed by the thin dashes depicts how odour magnitude would change in a case where concentration of odorant and irritant changed jointly. d, Same as c, but dichorhnic mixtures.

tions, the nasal septum insulated odorant from irritant but the sensory 'image' was essentially indistinguishable from that produced by a physical mixture¹⁰.

If the olfactory and trigeminal systems did not interact, the families of functions in Figs 1a, b and 2a, b would comprise

parallel lines. The converging trend reveals inhibitory interaction. Figures 1c, d and 2c, d depict the pattern of the inhibition for each perceptual component. Just as the perceived magnitude of carbon dioxide alone increased sharply with concentration (Fig. 1a, b), so did its ability to inhibit any accompanying odour (Fig. 1c, d). Similarly, just as the perceived magnitude of amyl butyrate alone grew gradually with concentration (Fig. 2a, b), so did its ability to inhibit irritation (Fig. 2c, d).

Figures 1 and 2 reveal a fundamental similarity between the physical (monorhynchic) and dichorhynchic mixtures. Because it rules out interference between odorant and irritant at the mucosal surface, dichorhynchic inhibition seems to establish the generality of the olfactory-trigeminal interaction beyond the stimuli used here. It also suggests that the interaction occurs in the brain.

The explanation of dichorhynchic inhibition must not ignore possible bilateral peripheral phenomena, such as changes in nasal patency¹. Such changes could arise from reflex vasodilation, which could increase intranasal resistance and diminish accessibility of the olfactory mucosa¹. To account for inhibition of odour, however, this mechanism would require the trigeminal response to precede the olfactory response consistently, an unlikely outcome in view of previous results^{11,12}. The trigeminal system generally seems to react to chemical stimulation slowly. The third experiment supported this view in a comparison of reaction times to carbon dioxide alone and amyl butyrate alone (Fig. 3a). Six subjects each made 400 responses over five sessions. Both odour and irritation exhibited intensity-dependent latencies, but at any given level of perceived magnitude the reaction to amyl butyrate always preceded that to carbon dioxide. The reaction to carbon dioxide was too slow to support the possibility that it generally inhibited odour magnitude through reflex changes in nasal patency.

The fourth experiment gave temporal advantage to the irritant to investigate whether peripheral factors are involved in the interaction between odour and pungency. Thirteen subjects (each making 96 judgements over three sessions) assessed the magnitude of amyl butyrate after inhalation of either 30 p.p.h. carbon dioxide or pure air. Each subject sampled the adapting stimulus (carbon dioxide or air) for 2 s followed immediately, on

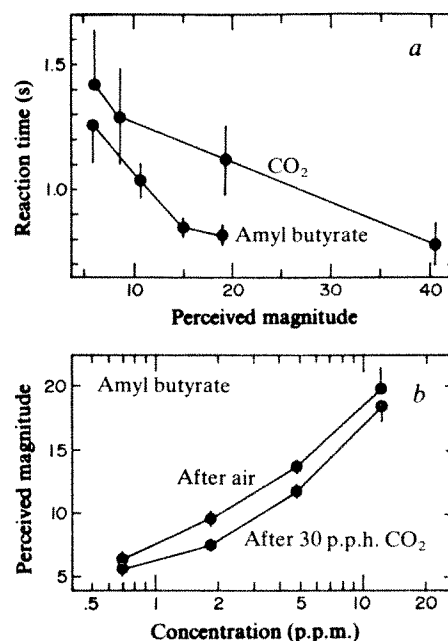


Fig. 3 *a*, Simple reaction time to four concentrations of amyl butyrate and to four concentrations of carbon dioxide plotted against the perceived magnitude of the stimuli. Data points are means (± 1 s.e.m.) taken across six subjects. *b*, Perceived magnitude of amyl butyrate smelt for 2 s immediately after 2-s inhalations of pure air (half the trials in each session, generally every other trial) or 30 p.p.h. carbon dioxide (other half of the trials). Data points are means (± 1 s.e.m.) taken across 13 subjects.

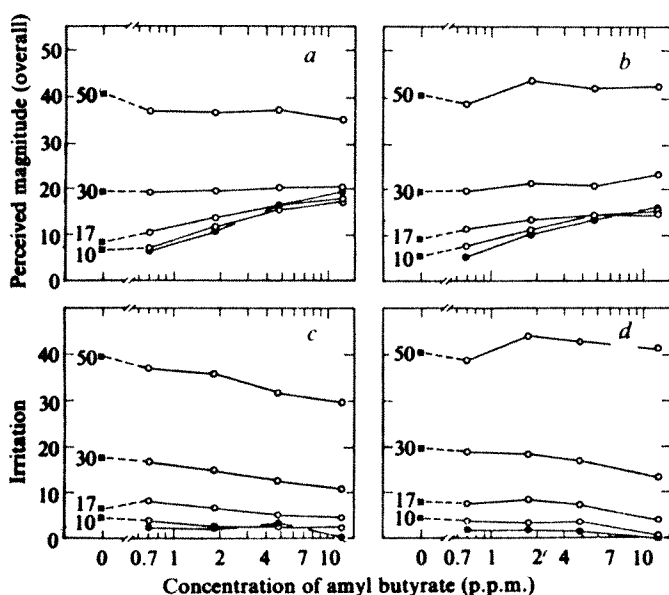


Fig. 2 *a*, Same psychophysical data as Fig. 1a, but plotted here against concentration of amyl butyrate. Symbols as follows: ●, amyl butyrate alone; ■, carbon dioxide alone; ○, physical mixtures. The concentrations of carbon dioxide (p.p.h.) are indicated at left for each. *b*, Same as *a*, but dichorhynchic mixtures. *c*, Perceived irritating component of carbon dioxide alone (■), amyl butyrate alone (●) and physical mixtures (○). *d*, Same as *c*, but dichorhynchic mixtures.

the same inhalation and through the same nostril, by amyl butyrate (2 s). In these conditions, carbon dioxide reduced odour intensity by about 12% (Fig. 3b). Simultaneous presentation had reduced odour intensity more than four times this amount (Fig. 2c, d). Accordingly, even in favourable temporal conditions, peripheral factors seem relatively impotent.

Because, if potent enough, an olfactory stimulus generally serves also as a trigeminal stimulus, olfactory-trigeminal interaction must be frequent. So far, the inhibitory interaction has revealed itself unambiguously only in the extreme case, such as where the inherent pungency of an 'odorant' outstrips odour magnitude, causing it to fall with increases in concentration^{7,12}. Figure 1c, d depicts how odour would first increase and then decrease with joint (co-varying) increases in the concentration of carbon dioxide and amyl butyrate. These non-monotonic functions could reflect how the odour of a single stimulus with both odour and pungency varies with concentration¹². Hints that such functions might emerge from central olfactory structures appear in observations that benign odorants will stimulate so-called background activity in the olfactory bulb, whereas 'sharp, unpleasant odors' will inhibit such activity^{13,14}. Walsh even noted a specific inhibitory effect of carbon dioxide on single units of the olfactory bulb (cats)¹⁵. These units also responded to the somaesthetic (trigeminal) stimulation caused by inhalation of odourless air.

Thus, we have confirmed, quantified and extended a century-old observation that pungency can diminish odour. We show: (1) a continuum of inhibition, (2) mutuality, whereby odour inhibits irritation and vice versa, (3) apparent generality beyond particular olfactory or trigeminal stimuli, and (4) a central neural site of interaction. The dimensions of the interaction may depend on various factors. Irritants will undoubtedly vary in their affinity for olfactory receptors. Even carbon dioxide, although odourless, might conceivably cause some unspecified modulation of olfactory receptors. Manner of trigeminal activation may also have a role; perhaps trigeminally mediated warmth, cold or pressure can modulate odour. Finally, the timing of olfactory and trigeminal activation may be involved. Differences in speed

of activation from odorant to odorant and irritant to irritant, not necessarily revealed best by simple reaction time, may govern any contribution from peripheral factors. Irrespective of the mechanism of the interaction, our results suggest a need for special attention to odorous and irritating contaminants in the workplace. An irritating gas may mask the presence of useful olfactory signals (such as, warning agents) and an odorant may mask the possibly corrosive vapours (for example, inorganic acids) that often form the stimulus for the trigeminal nerve.

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Illusory reversal of extrafoveally perceived displacement

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It has long been known that the temporal characteristics of human peripheral vision differ markedly from those of foveal vision. Slowly moving peripheral stimuli, for example, can give rise to dramatically exaggerated estimates of their displacement^{1,2}. More recently, Thorson *et al.*^{3,4} have shown that where two spots are flashed in sequence to peripheral vision with an interflash interval of 50 ms, a sensation of movement can be induced even when the spatial separation of the spots is below the static acuity threshold. These observations fit with several others^{5,6}, suggesting that the visual system uses separate channels for signalling 'motion' as distinct from 'change of location', and that in some circumstances the integration of motion signals may make a dominant contribution to the perception of displacement. I now report a striking illusion which seems to reinforce and extend this conclusion.

This illusion is easily demonstrated and quantified using a double-beam cathode-ray oscilloscope with a non-persistent screen in a normally lit room. If one spot (or line) on the screen is deflected instantaneously through 1° or 2° and allowed to return more slowly to its original position, for example by alternate (1 s⁻¹) exponential-spike waveforms of 25 ms time constant and opposite polarity (Fig. 1a), then in foveal vision its motion is perceived more or less veridically. Viewed 3° or 4° extrafoveally, however, the appearance is surprisingly different. The displacement perceived is actually in the opposite direction to the real, the apparent motion being as shown in Fig. 1b. The illusion is readily quantified⁷ by imposing an RC-filtered step deflection of this form on the second cathode-ray tube beam, and adjusting its amplitude and time constant until the motion of the second

spot or line, when viewed foveally, seems to match that of the first (perceived extrafoveally). Detailed measurements are still in progress and will be reported elsewhere, but for photopic stimuli viewed at retinal eccentricities of a few degrees, the motion perceived is just as if the rapid displacements had not occurred. Furthermore, if fixation is changed so that both stimuli are viewed extrafoveally, their perceived motions still seem to match.

It thus seems that in these conditions only the slow phases of the image motion are signalled by the extrafoveal system to the centres that mediate motion perception. As the resting position of the spot (between spikes) remains physically unchanged (Fig. 1a), but is perceived as displaced alternately by an amount $\pm x$ (Fig. 1b), extrafoveally perceived location seems here to be computed entirely by integration from motion signals, rather as in an 'inertial navigation' system. (The rising phase of the spike, which takes only a few microseconds, is presumably too rapid to stimulate any physiological 'motion detectors' (ref. 8).)

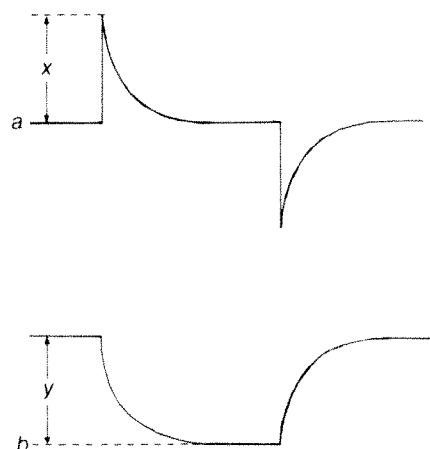


Fig. 1 a, Waveform of deflection of extrafoveally viewed spot (spike deflections $\sim 1^\circ$, 1 s⁻¹). b, Waveform of perceptually matching deflection of foveally viewed spot. For perceptual match, $y = x$ and time constants are equal.

Note that the displacement x (Fig. 1a) was here much larger than the static acuity threshold for the extrafoveal region stimulated. When a square wave of the same amplitude was substituted for a, the spot motion was clearly perceptible. The present illusion is thus distinct from, and in a sense complementary to, the Thorson effect^{3,4} described above.

Further experiments have shown that at scotopic light levels (with only the retinal rods active) the perceptual match between the foveal and extrafoveal stimuli of Fig. 1 breaks down. This suggests that the computation of extrafoveal displacement on the basis of motion signals is primarily a function of the cone system. In this connection it may be significant that it is normally only at scotopic light levels (when the cone system is inactive) that illusory instability of the perceived world is seen during saccadic eye movements. The findings reported here suggest that at photopic levels the processing of extrafoveal information may be undisturbed by the image displacements produced by normal saccadic exploratory movements.

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Young kittens can learn complex visual pattern discriminations

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Kittens begin to display visually elicited responses shortly after eye opening. The pupillary reflex¹, optokinetic response^{2,3}, visual placing³⁻⁵ and avoidance of the deep side of the visual cliff^{3,5-7} all appear between the second and fifth weeks of life, and evidence of visual learning in homing behaviour has been described at the same early age⁸. However, an attempt to elicit pattern preferences in kittens of this age (usually considered the easiest way to demonstrate discriminatory behaviour in immature organisms⁹) was unsuccessful, evidently because such kittens show little spontaneous visual interest^{10,11}. This is surprising in view of electrophysiological findings which emphasise the importance of the fourth to sixth weeks in the development of cortical feature-analysing mechanisms¹²⁻¹⁴, perhaps the stimulus-seeking methodology of Dodwell *et al.*^{10,11} is inappropriate for the visual modality at such an early age. Recently, visual acuity measurements have been reported for kittens as young as 30 d of age using a contour versus no-contour discrimination on a modified Lashley jumping stand^{15,16}. With this technique we have now trained kittens of under 50 d of age to perform more complex pattern discriminations.

In the jumping stand, kittens have to jump from the lip of a short tunnel on to one of two stimulus surfaces, a drop of 15–20 cm. The surfaces are trapdoors, and an incorrect choice results in a fall of about 45 cm. Correct choices are rewarded with food and petting. An average kitten is able to perform this task adequately at 28–30 d of age, thus setting a lower age limit to its use. To accustom the animals to the jumping stand and to obtain an initial estimate of their learning ability, all kittens are pretrained on a white/black discrimination (white positive). The 20 kittens in our experiment all exhibited either an immediate preference for white, or learning within 80 trials. Accustoming the animal to jumping, black/white training, and gradually increasing the jumping height to a maximum of 15–20 cm, takes 5–10 d, thus placing the onset of pattern discrimination training between the 35th and 40th day of life.

Different groups of kittens of this age were trained to discriminate each of the three pattern pairs shown in Fig. 1. These stimuli constitute the three basic orthogonal orbit-pairs of Hoffman's Lie transformation group theory of pattern perception (ref. 17 and P.C.D., unpublished) and were chosen for that reason; the theory predicts that they will be the easiest patterns to discriminate, particularly for an immature animal¹⁷. However, patterns similar to pairs *a* and *b* have been widely used in the study of visual perception (refs 18–20 and P.C.D., unpublished) pair *b* in particular having been generally regarded as the simplest possible pattern discrimination because its resolution involves only orientation detection²⁰. Pair *c* (families of rectangular hyperbolae) have not been used before. The stimuli were photographic enlargements of these patterns mounted on 32-cm² white cards and plasticised. The black contours were 1.5 cm wide, thus subtending visual angles of slightly more than 4° of arc when viewed from a height of 20 cm. This should be well within the visible range for kittens at 35 d of age¹⁵. Each stimulus pair was matched as closely as possible for overall luminous flux.

Kittens were given 40 training trials per day until the criterion of 27 of 30 consecutive trials correct was met. The number of trials (excluding the final 30) and errors to criterion for the three groups are shown in Table 1, with the age at which criterion was reached. Clearly, kittens are able to master each of these problems very early in life. For comparison, we have included data for problem *a* from seven older kittens (mean age at criterion 80 d), some of which had previously learned other problems and some of which were experimentally naive. Both young and older kittens showed similar performance, so apparently the young group made use of visual pattern information in a normal manner throughout the training period, and learning was not impaired by visual or motor immaturity. This is probably true also of problems *b* and *c*, but we do not yet have sufficient data to confirm the point. The results in Table 1 suggest that for very young kittens the problems increase in difficulty from *a* to *c*. We are now investigating whether or not this is an age-dependent phenomenon.

Solution of each discrimination problem was followed by a series of transfer tests. These were designed to rule out the involvement of various extraneous cues and to determine to which aspects of the stimulus configuration the animals were responding. Transfer tests were typically administered by a different investigator from the one who administered the training trials; in no case did performance deteriorate as a result of this change. On the basis of the perfect or near-perfect transfer scores obtained, such factors as olfactory cues, auditory and visual cues associated with locking the trapdoors, and visual cues specific to the particular pair of stimulus cards used in training could be ruled out. A full description of the methodology and results of these tests will be published elsewhere.

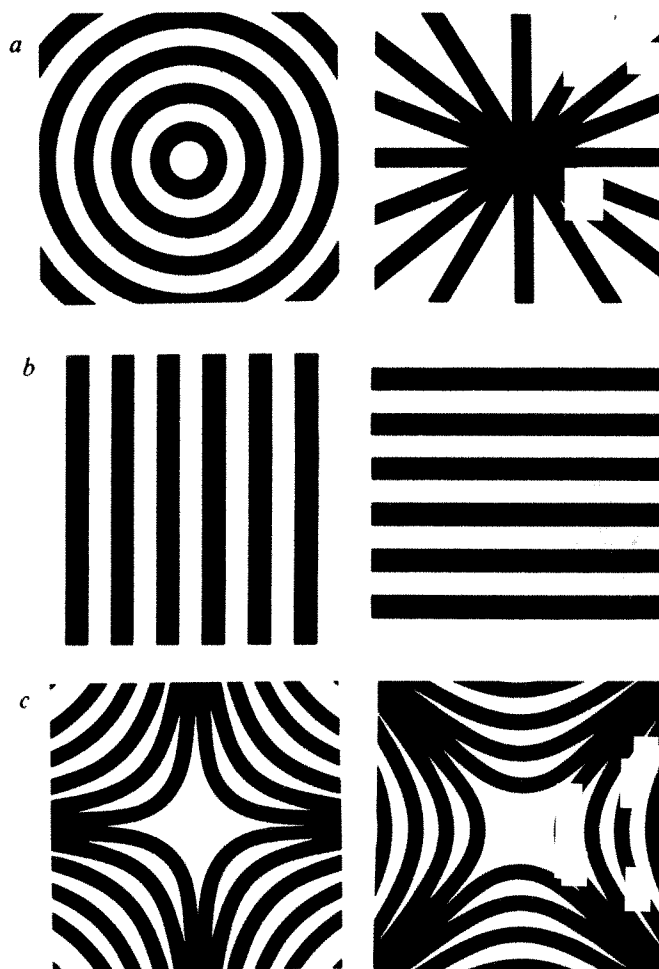


Fig. 1 The three pattern pairs derived from Hoffman's Lie transformation group theory.

Table 1 Summary of learning scores

Problem	No. of kittens	Trials to criterion	Errors to criterion	Age at onset of training (d)	Age at criterion (d)
a	9	115 (40–218)	53 (19–99)	38 (35–40)	43 (38–47)
b	4	271 (97–435)	113 (49–225)	38 (35–40)	48 (38–58)
c	4	327 (163–562)	138 (79–239)	38 (33–43)	48 (46–51)
a (late)	7	119 (42–187)	53 (21–89)	76 (45–120)	80 (48–123)

Results given are the mean; the range is given in parentheses. Group *a* (late) consisted of the four kittens from problem *b* group, one kitten from problem *c* group, and two experimentally naive kittens.

For problems *a* and *c* the most crucial test was the contrast-reversal test, in which the stimuli were negative photographic enlargements of the same original patterns. The contrast relationships within each figure were thus reversed, as was the direction of any overall luminance difference between figures. This test was given to 14 of the 16 animals trained on problem *a* and three of the four kittens trained on problem *c*. Contrast-reversal test trials were intermixed with training trials on the original stimulus pair. Food reward followed all test trials regardless of the animal's response whereas, on training trials, the reinforcement contingencies described above were used. The range of transfer scores fell between 7/10 and 10/10 correct, with a mean of 9.0. This constitutes fairly strong evidence that the kittens were not solving the problems on the basis of overall luminous flux or local brightness cues, thus suggesting that they coded the features of the patterns as a whole, and independently of contrast.

We have thus demonstrated that kittens between 30 and 50 d of age can be taught arbitrary associations between certain visual patterns and positive and negative rewards. This seems almost paradoxical in view of the earlier finding that kittens show little, if any, spontaneous interest in visual stimulation at this age¹⁰. The apparent anomaly can probably be accounted for in terms of the extrinsic rewards associated with our present training procedure. Nevertheless, the findings reported here open the way for investigating the ontogeny of visual pattern analysis under much tighter experimental control than is provided by the naturalistic situations in which visual learning has previously been described in very young kittens⁸. Our initial results indicate that at the peak of the so-called 'critical period', when neurones of the visual cortex are most sensitive to environmental modification^{21,22}, kittens are able to extract and use visual pattern information in a rather sophisticated manner, albeit from patterns which, from a certain theoretical point of view, are the simplest ones available.

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A demonstration of navigation by small rodents using an orientation cage

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The ability to 'home' after displacement has been demonstrated for several species of small mammals¹. Whether such homing is accomplished by random scatter², familiarity with a large area^{3,4} or some kind of navigation mechanism⁵ is still unknown. The ability to navigate (to determine the direction that will lead the animal to the required destination⁷) has been shown, for example, by releasing displaced rodents on snow and analysing their tracks⁶ and by analysing for homeward bias the directions from the release point of traps in which displaced individuals were subsequently recaptured¹. Such techniques are limited in application. Moreover, the latter technique is susceptible to differential avoidance of traps by animals travelling in different directions³. It is potentially more useful to retain displaced individuals in an orientation cage and to examine their movements within the cage for evidence of orientation in the homeward direction. However, previous attempts using various types of orientation cages have yielded only negative results^{8–10}, except, surprisingly, when the displaced animals could not see their natural surroundings. We present the first positive evidence that small rodents will navigate while retained in an orientation cage, even when able to see their surroundings. Indeed, we show that these visual cues are important in navigation and suggest why orientation cages have previously led to negative results.

Our main experimental species was the European wood-mouse, *Apodemus sylvaticus* although the yellow-necked mouse, *A. flavicollis*, and bank vole, *Clethrionomys glareolus*, were occasionally tested when available. In all, 19 individuals were used (*A. sylvaticus*, ten males and four females; *A. flavicollis*, one male; *C. glareolus*, two males and two females) in a total of 116 4-minute test periods on 72 displacement occasions. Although mainly nocturnal, *Apodemus* spp. are known also to be active during the day, particularly juveniles (unpublished observations and refs. 11 and 12). It was presumed, therefore, that if they had navigational ability, these species, like the more diurnal *C. glareolus*, ought to be able to make use of cues available during the day as well as the night. It was also anticipated, and borne out by this work, that a nocturnal animal would show a strong homing response during daylight.

All experiments were conducted between 10.00 and 17.30 h GMT between 11 and 25 July 1979 at Woodchester Park Field Centre, Gloucestershire. Animals were caught in Longworth traps, either in a meadow (12 traps in a 110-m line) or in woodland (96 traps in a 70 m × 110 m grid described by Yalden¹³). The woodland was situated above the meadow on the same north-west facing slope of an enclosed V-profiled valley. The orientation cage designed for this work and the experimental procedure are described in Fig. 1 legend. After testing, the animal was either returned to the point of capture or released from the final displacement site and the vanishing point direction (the direction of last observed position from the test site) recorded. Vanishing point direction (up to 30 m from the test site) was often very different from the initial direction of movement after release, which, as found by other authors⁷, was usually aimed at the nearest cover.

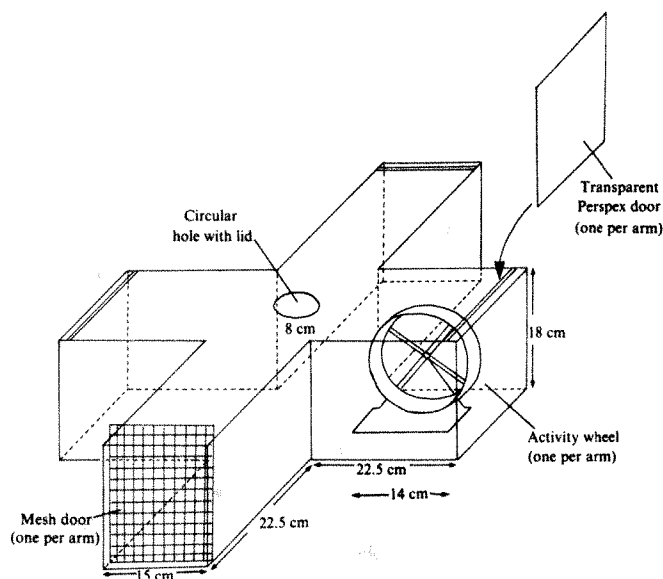


Fig. 1 The orientation cage was a cross-shaped Perspex chamber with a black opaque floor and transparent sides and top. A mesh door at the end of each arm of the cross could be raised to allow the animal to exit. An activity wheel was positioned in each arm as part of a separate experiment but which incidentally (and importantly) provided shelter. After capture, each individual was identified and weighed, then placed in the apparatus through a circular hole in the centre of the lid. An opaque black polythene sheet was used to cover the apparatus during transport to each of the displacement sites where an area of level ground was selected. The apparatus was then positioned so that the four arms were directed towards magnetic north, south, east and west. The polythene sheet was then removed and the observers withdrew to a vantage point ~5 m away. The experimental animal was allowed to visit three of the four arms before recording began. Total time spent in each of the four arms was then recorded, but periods of grooming, which were often long, were excluded. The danger of subjectivity thus introduced was reduced by the fact that two observers were always present, each with a stopwatch. In practice, the beginning and end of grooming periods were clear-cut and exclusion was considered preferable to lumping the behaviour with periods of movement and environmental monitoring. Recording ceased after 4 min (excluding grooming time). Whenever possible, this experiment was repeated with the same individual at four displacement locations, each varying in distance and compass direction from the central point of capture. At each displacement site, the direction and distance of the trap, observers and nearest vegetation/cover were recorded and also weather conditions and time of day. The influence of smell of previous occupant was controlled by: (1) maintaining the same arm of the apparatus in a constant compass direction during each day's experiments; (2) varying the compass sector from 'home' (see Fig. 2) of the first test location for successive individuals; and (3) testing each individual at locations in more than one compass sector from home. Once navigation in the equipment had been demonstrated, possible visual and olfactory mechanisms were examined. At each displacement site various experiments were conducted involving the use of a visual barrier (a cardboard screen, 60 cm high and 35 cm radius, with a small hole 1 cm in diameter for observation) which surrounded the apparatus, and a partial olfactory barrier (perspex doors which were slotted in alongside the mesh doors).

Statistical analysis was carried out in two stages as described by Batschelet^{14,15}. Time spent in each of the four arms of the orientation cage was used in a first-order analysis to calculate the mean vector (r, θ) for each 4-minute test (Fig. 1). The mean angles were then subjected to second-order analysis¹⁵. Non-uniformity was examined by Rayleigh's Z -test¹⁴. Stephens' exact test and Wheeler and Watson's parametric two-sample test¹⁴ were also used, as indicated in Table 1 and Figs 2, 3.

In displacement experiments, the interpretation of navigational ability is very much dependent on the experimenters making the correct guess concerning the animal's intended destination. We had to presume that the animals were residents and that the point of capture was located centrally within the individual's home range, to which it would want to return after

being displaced. Hence, point of capture was taken to be the location of home towards which the animal would orientate while retained within the apparatus, and statistics are presented with trap direction designated as 0° . However, this is a particularly stringent criterion as the directions of the trap and intended destination are unlikely to be identical. Nevertheless, our results (Table 1) show that on average the mean orientation in the apparatus and the direction of movement after release are not significantly different and that vanishing point direction is not significantly different from the direction of the trap. We conclude that the mean orientation in our cage reflects the direction in which the animal would travel (after seeking cover) if unrestrained and that this direction is towards home.

The data were analysed with respect to a number of potentially confounding variables and the results are summarised in Table 1. The number of occasions an individual was caught in a Longworth trap does not influence performance. In contrast, the number of previous uses in the test apparatus does. Individuals used for the first and second times show a preferred orientation near to 'home' direction. However, for some reason as yet unknown, individuals being used on their third occasion showed uniform orientation.

The preferred orientation was not influenced by the position of the observer or the nearest vegetation cover, but was affected by weather conditions. At wind speeds less than force 3 (Beaufort Scale) orientation is most marked when the Sun is visible but is non-uniform even when not. However, winds of Force 3 or greater disrupt orientation with respect to home,

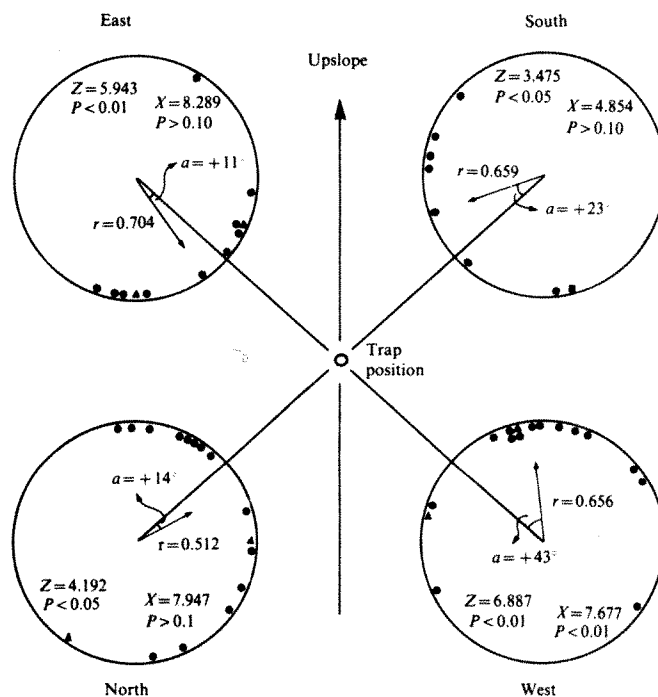


Fig. 2 Effect of displacement direction on mean orientation was shown by lumping data for displacements in four compass sectors around point of capture (trap position). All displacements 6–80 m from the trap are presented (excluding tests: (1) in wind speed of Force 3 or greater; (2) involving individuals being used on their third or more occasion; and (3) with a visual barrier). Each point (●, *Apodemus sylvaticus*; ■, *A. flavicollis*; ▲, *Clethrionomys glareolus*) represents the mean angle (θ , first-order analysis) for an individual during a 4-minute test period. Mean angle is expressed as positive or negative (+ or -) deviation from direction of trap of capture. All second order mean vectors (arrows) are significantly non-uniform. Mean vectors from N, E and S are not significantly different from trap direction (Stephens' exact test). From the W sector, the mean vector, although towards the trap, is significantly different from the trap direction. However, the inclusion of short-distance displacements (6–20 m) imposes a positive bias to the mean vector (see Fig. 3), an effect that possibly may have been compounded by some unslope orientation from this sector. We conclude that our animals were showing navigation in the orientation cage in the conditions and at the distances tested.

Table 1 Effect of various factors on orientation performance in the test apparatus: summary of second-order analyses

Variable	No. of tests (n)	Mean angle (θ°)	Mean vector (r)	Z	X	F	Exclusions*
<i>Home = 0°</i>							
Comparison of vanishing point direction (VP) with mean direction (MO) in test apparatus							
VP	13	+28	0.511	3.398†	5.868}	0.043	A, E
MO	13	+21	0.510	3.381†	6.190}		
No. of times caught:							
1 or 2	29	+19	0.531	8.172‡			B, C, D, E, F
3-16	20	+31	0.673	9.056‡			
Performance on successive trials:							
first use	36	+24	0.579	12.077‡			B, C, D, F
second use	13	+25	0.604	4.740‡			
third use	13	-74	0.220	0.627			
Effect of weather:							
Wind speed < force 3							B, C, E, F
with Sun	32	+25	0.631	12.737‡			
Sun obscured	7	+44	0.758	4.024†			
Wind speed > force 3	12	+49	0.183	0.403			
with Sun	5	-130	0.085	0.036			
Sun obscured	7	+49	0.375	0.983			
Effect of habitat:							
open meadow	24	+21	0.567	7.715‡			B, C, E, F
woodland	28	+26	0.641	11.488‡			
Sex							
male	26	+29	0.560	8.147‡			B, C, E, F
female	26	+17	0.645	10.822‡			
Reduced cues due to							
opaque screen	8	+15	0.274	0.600			B, C, D, E
Perspex doors	11	-3	0.572	6.291‡			
Observer direction = 0°	68	172	0.075	0.386			D, E, F
Nearest vegetation = 0°							B, C, E, F
trap distance							
6-80 m	46	49	0.176	1.421			
>80 m	5	89	0.289	0.419			
Upwind = 0°	14	78	0.475	3.164†			F, G

* A, animals returned to trapping site for release; B, test <6 m from trap; C, tests >80 m from trap; D, tests at wind speeds force 3; E, individuals on third time trial or more; F, tests with opaque screen; G, tests at wind speeds < force 3.

† $P < 0.05$.

‡ $P < 0.01$.

Z, Rayleigh's test; X, Stephens' exact test; F, Wheeler and Watson's 2-sample test.

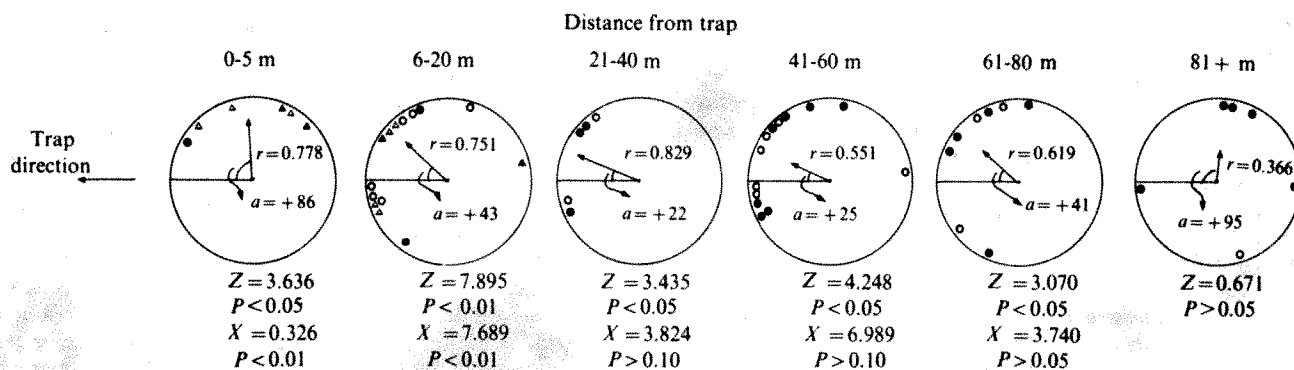


Fig. 3 The accuracy of orientation was influenced by displacement distance. Displacements in all directions from the trap are shown, excluding tests influenced by wind and previous usage as described in Fig. 2. Diagrammatic conventions are as in Fig. 2 except that open symbols denote females and closed symbols males. All second-order mean vectors (arrows) for distances less than 80 m are significantly non-uniform. At short distances (<20 m), mean vectors are significantly different from trap direction (Stephens' exact test), probably reflecting the difference between the directions of the trap and intended destination, although the meaning of the clear positive deviation is unknown. At greater distances a positive bias is still evident, though not significantly different from trap direction. Beyond 80 m, orientation is not significantly non-uniform, but sample size is small.

whether the Sun is visible or not. Instead, at these wind speeds the animals orientated at right angles to upwind, thereby spending more time in the most sheltered arm of the apparatus.

Final demonstration of the occurrence of navigation towards home in the apparatus was achieved by examining mean orientation from four different compass sectors relative to trap position (Fig. 2). Navigation has been demonstrated for distances up to 80 m from point of capture (Fig. 3) which is nearly three times the distance of half the home range length (0.5×56.6 m) of male *A. sylvaticus* at Woodchester in summer¹⁶. However, 80 m is far less than the distance from which homing has been demonstrated for *Apodemus* spp. elsewhere (59% return from 250 m,

17% return from 750 m etc.)¹. This could imply that navigation is not used at these longer distances and that homing is achieved by other mechanisms such as random search (see Bovey¹ for discussion). However, our experimental animals were limited to cues available at the particular displacement site and had to reach a decision concerning home direction within a few minutes. Further experiments at longer distances are planned with the animals being allowed more opportunity to acquire and process information as to their location.

Other factors were examined (Table 1) but were found to have no significant effect on the direction of orientation. These factors included whether the displacement site was in open meadow or

woodland and time of day (the latter is not shown in Table 1). Nor were any overall differences found between males and females (see also refs 4 and 17). However, when displacement distance was considered, there was an indication that orientation was most accurate for females at distances less than 40 m but for males at distances between 40 and 60 m (Fig. 3).

An opaque screen surrounding the apparatus disrupted orientation (Table 1), supporting the suggestion that woodmice use visual cues for navigation^{18,19}. The addition of Perspex doors (in the absence of a screen) (Fig. 1) did not disrupt orientation (Table 1). However, all 11 individuals thus tested showed a decreased bias (that is, first-order r value) towards the mean angle compared with the bias when the Perspex doors were removed. This was due to an increase in the amount of movement in the apparatus when the doors were in position, all arms being visited more frequently and evenly. During this movement, the animals often climbed the bars to sniff at any gaps still remaining. Vision thus seemed to be the major sense involved in navigation but additional information may have been sought from olfaction.

Our experiments have shown that in certain conditions small rodents will orientate toward home while in an orientation cage and will do so at distances up to at least three times the radius of their home range. Visual and perhaps olfactory senses both seem to be involved. We suggest that our experiments produced

positive results because, unlike previous orientation cages, ours offered the animal shelter from the wind and the opportunity to take cover (under the running wheels) should it feel threatened. As a result, the animals orientated toward home rather than toward the nearest cover, the main cause of negative results in previous experiments.

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Oestrogens regulate divergent effects of prolactin in the ovary

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Prolactin exerts well established stimulatory effects both *in vitro* and *in vivo* in the rat ovary¹. However, in the ovary of higher mammals, prolactin action is not well characterised, and may differ from that observed in rodents². For example, in human clinical conditions of physiological or pathological prolactin excess, ovarian function is depressed³, and in isolated, human granulosa cells *in vitro*, prolactin seems to inhibit progesterone production⁴. To examine direct prolactin effects in the higher mammalian ovary, we have used an *in vitro* porcine granulosa-cell model⁵. In this system, prolactin action is bipotential, depending critically on the degree of granulosa-cell differentiation attained *in vivo*. Prolactin suppresses steroid production by cultured granulosa cells isolated from small (1–2 mm), immature follicles, but stimulates progesterone secretion by granulosa cells collected from large (>6 mm) mature follicles⁶. The present studies show that oestrogens may play an important part in regulating these divergent actions of prolactin in the ovary.

To investigate potential modulation of prolactin action by oestrogens, we maintained granulosa cells collected from immature porcine follicles in monolayer culture for 10–12 d. As previously observed, prolactin alone produces inhibitory effects (Fig. 1a). However, the continuous administration of oestrogens to granulosa cells produces a biphasic response and a striking oestrogen–prolactin interaction that is time dependent. Early in culture (days 0–2) oestradiol alone exerts dose-dependent inhibition of progesterone production. Oestradiol decreases the total mass of progesterone contained in cells and medium (data not shown), indicating a reduction in actual steroid synthesis rather than in steroid secretion alone. The range of inhibitory concentrations of oestrogens used *in vitro* encompassed that observed in porcine follicular fluid comprising the *in vivo* micro-milieu of immature to increasingly mature granulosa cells⁶. When prolactin is added to submaximally inhibiting concen-

trations of oestrogen during this initial culture period, progesterone secretion declines further. Thus, both oestrogen and prolactin exert inhibitory effects early in culture and these effects are additive at submaximal steroid concentrations.

After 48 h of continued oestrogen exposure, cultured granulosa cells exhibit a reversal in responsiveness to oestrogen effects, and show enhanced progesterone secretion. From day 2 onward in culture, oestradiol stimulates steroidogenesis, and, more strikingly, produces a 'switch' in prolactin action from inhibitory to stimulatory (Fig. 1b). The interaction of oestradiol and prolactin is synergistic in enhancing progesterone production on all days throughout culture after day 2 ($F > 20$, $P < 0.01$ by 2-way analysis of variance). The oestrogen effects exhibit steroid specificity. The stimulatory actions of oestrone ($1 \mu\text{g ml}^{-1}$) and 17β -oestradiol ($1 \mu\text{g ml}^{-1}$) were quantitatively similar, at 7.4 ± 0.7 and 8.7 ± 0.6 ng progesterone per 10^6 cells per 48 h, respectively, with control cultures 2.8 ± 0.3 (mean \pm s.e.m., $n = 4$, $P < 0.01$ by one-way analysis of variance). However, the less potent oestrogen, oestriol ($1 \mu\text{g ml}^{-1}$), exerted no significant effect (2.1 ± 0.25), and the anti-oestrogen, nafoxidine hydrochloride (10^{-7} M), produced slight but insignificant suppression of steroid secretion (1.6 ± 0.2). Although oestrogens are mitogenic for granulosa cells *in vivo* and *in vitro*⁷, oestrogenic effects cannot be accounted for by simple alteration in cell number. 17β -oestradiol ($1 \mu\text{g ml}^{-1}$) increased the number of cells per culture by only $28 \pm 9\%$ above control ($n = 18$ experiments). Thus, when progesterone accumulation is corrected for cell density, both the acute inhibitory and the delayed stimulatory changes in progesterone secretion persist at various times in culture (Fig. 2).

In addition to modifying granulosa-cell responsiveness to prolactin, oestrogens seem to exert important intrinsic effects on steroid secretion by the ovary. *In vivo* and *in vitro*, oestrogens in conjunction with gonadotropins facilitate follicular maturation and steroidogenesis in several species^{7–9}. However, inhibitory effects of oestrogen on granulosa cell progesterone secretion have also been described^{10–14}. The present study demonstrates that the action of oestrogen may be bipotential, and critically dependent on the duration of hormone exposure. Acute oestrogen administration produces inhibitory effects, concordant with observations by Haney *et al.* in porcine granulosa cells¹⁰, and with work by other investigators in luteal tissue of the cow¹¹, ewe¹², monkey¹⁴ and human¹⁴. However, our findings also reveal delayed, but sustained stimulatory effects of oestrogen. A similar response to chronic oestrogen exposure in

replicating cultures has been reported by Bernard *et al.* in rat¹⁵ and by Goldenberg *et al.* in pig granulosa cells *in vitro*¹⁶, but was not observed by Thanki and Channing when cultured porcine granulosa cells were maintained in serum-restricted conditions of stationary growth¹⁷. These observations suggest that factors related to cell proliferation must also be considered in the expression of oestrogen action in the ovary.

The directional reversal of prolactin action induced by oestrogens *in vitro* is similar to that which occurs spontaneously during maturation of porcine ovarian follicles *in vivo*^{5,18}. Granulosa cells of the pig may differ in this regard from those of the human (in the latter, prolactin seems to suppress progesterone secretion regardless of follicle size)⁴. This difference may reflect species characteristics, or exemplify the requirement to select mature, 'pre-ovulatory' follicles. Such follicles are exposed to high endogenous oestrogen concentrations *in vivo*, and are required in our system to demonstrate stimulatory effects of prolactin *in vitro*. Because intrafollicular oestrogen

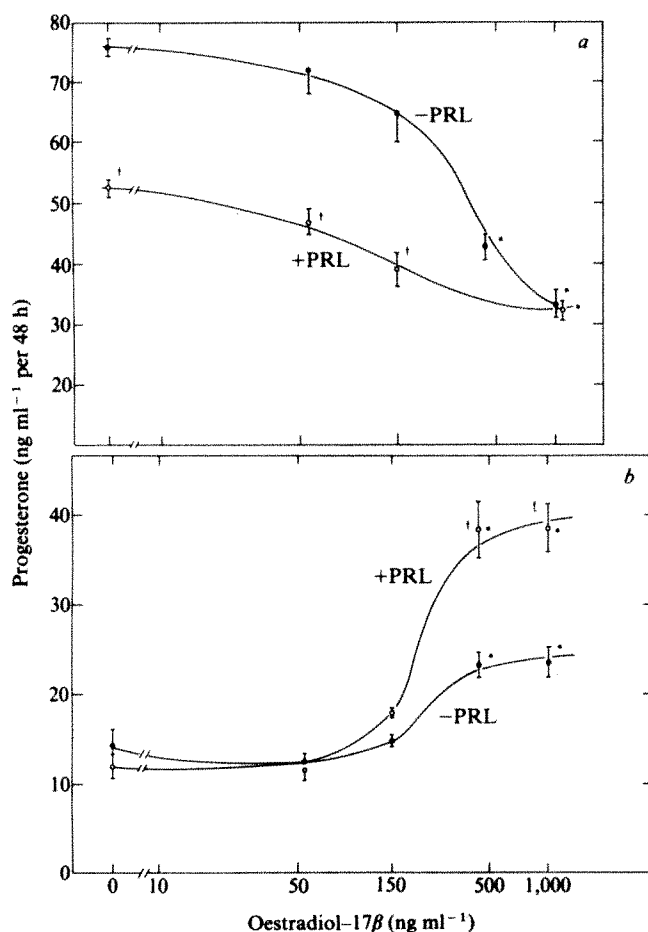


Fig. 1 *a*, Day 2, Acute oestrogen administration to cultured porcine granulosa cells suppressed progesterone production in a dose-dependent manner. Prolactin (PRL) (100 ng ml⁻¹) further inhibited progesterone accumulation in the presence of sub-maximal oestradiol concentrations. *b*, Day 4, Continuous oestrogen exposure enhanced progesterone secretion and reversed the inhibitory action of prolactin. The interaction of oestrogen and prolactin was synergistic in augmenting steroid accumulation ($F > 20$, $P < 0.01$ by 2-way analysis of variance). Granulosa cells were aspirated from 1–2-mm follicles of porcine ovaries and maintained for 2–10 d in monolayer culture (medium 199, 10% fetal calf serum, bicarbonate buffer and antibiotics⁵). Quadruplicate cultures were treated with increasing concentrations of 17 β -oestradiol, in the presence or absence of prolactin (100 ng ml⁻¹, NIH-oPRL-S12). Spent medium was removed at 48-h intervals for the subsequent determination of progesterone by radioimmunoassay²³. Data are mean \pm s.e.m. ($n = 4$). * $P < 0.01$ compared with basal (no oestrogen, no prolactin); † $P < 0.01$ compared with oestrogen treatment without prolactin.

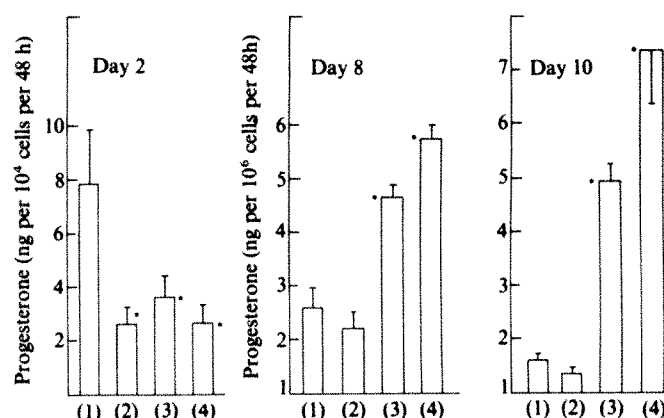


Fig. 2 Acute inhibitory and delayed stimulatory interactions of oestrogen and prolactin on steroidogenesis in cultured porcine granulosa cells. Data are expressed as the mass of progesterone (ng) produced per 10⁴ cells (day 2), or per 10⁶ cells (days 8 and 10). Cultures were treated with ovine prolactin (100 ng ml⁻¹) (column (2)) or 17 β -oestradiol (1 μ g ml⁻¹) (column (3)) or both (column (4)), for 2, 8 or 10 days before enumeration of cell density by electronic (Coulter) particle counting⁵. Column (1) shows control levels. * $P < 0.01$ versus control.

concentrations increase 20–100-fold during follicular maturation *in vivo*^{19,20}, our observations of oestrogenic effects in cultured porcine granulosa cells suggest that oestrogens may govern the physiological responses of these cells to prolactin *in vivo* as well as *in vitro*.

The mechanism of the potent interaction between oestrogens and prolactin is a challenge of these studies. We previously demonstrated that porcine granulosa cells freshly collected from small immature follicles exhibit high concentrations of specific, high-affinity prolactin binding sites¹⁸, which are maintained during continuous monolayer culture⁵. Oestradiol seems to double prolactin binding by these cultured porcine granulosa cells²¹. However, a simple increase in prolactin receptor concentrations would fail to explain the directional reversal of prolactin effects in oestrogen-treated cells. Conversely, prolactin modification of cellular oestrogen binding must also be considered. For example, in rat luteal tissue *in vivo*, prolactin induces oestradiol receptors²², and is synergistic with oestrogen in enhancing steroidogenesis. However, regulatory changes at other cellular loci of hormone action will also require further examination, as the intracellular mechanisms subserving the actions of prolactin and the steroidogenic effects of oestrogens are poorly understood. Further investigation of the hormonally responsive ovarian cells used in the present studies should allow a more comprehensive understanding of the cellular basis for these critical hormone interactions in the mammalian ovary.

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Axons from CNS neurones regenerate into PNS grafts

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Axons in the peripheral nervous system (PNS) and central nervous system (CNS) form sprouts after injury^{1–3}. Elongation of regenerating axonal sprouts has been observed as the exception within the adult mammalian CNS but is the rule in the PNS of mammals as well as in the CNS of some fish and amphibians⁴. The relative importance of intrinsic neuronal properties and axonal environment in determining the extent of axonal regrowth is unknown⁵. Neuroglial cells, nerve growth factor and target tissues such as smooth muscle are known to influence neuronal responses to injury^{6,7}. Here we have examined the capacity of transected axons originating in the CNS to regrow into nerve grafts containing Schwann cells.

The surgical technique used, a modification of that previously described^{8,9}, was carried out in young adult Sprague–Dawley rats. In the first group of 16 animals, a 5-mm segment of the midthoracic spinal cord was removed and an autologous sciatic nerve graft was inserted sub-pially between the two stumps of the spinal cord. One to four months later, the animals were anaesthetised and perfused. The grafts and adjacent spinal cord tissue were examined by light and electron microscopy. The perineurium surrounding the common peroneal and posterior tibial fascicles of the sciatic nerve was clearly identifiable in cross-sections through the middle of the graft and contained many myelinated or unmyelinated axons ensheathed by Schwann cells. The ultrastructural appearance of each graft was similar to that of a regenerated peripheral nerve. The grafts were in gross continuity with the proximal and distal spinal cord stumps. At the spinal cord–graft interfaces, small cysts were frequently found but were rarely greater than 1 mm in diameter. In electron micrographs, the marginal spinal cord tissue was seen to contain many astrocytic processes and to be surrounded by a basal lamina. Small, irregular dome-like neuroglial structures were observed to protrude towards the graft at the ragged edge of the spinal cord tissue. These protuberances resemble structures seen at the interface of optic nerve grafts and peripheral nerves¹⁰ and also at the normal dorsal root entry zone¹¹. Occasional fibres were found where the axon was surrounded by peripheral myelin (periodicity 15.0 nm) and Schwann cell cytoplasm on one side of a node of Ranvier and central myelin (periodicity 13.6 nm) and astrocytic processes on the other. From these morphological observations we conclude that some axons crossed the border between the graft and spinal cord, although the direction in which they grew could not be determined.

Because of the potential ability of axons in the dorsal and ventral spinal roots to innervate such grafts, all roots crossing the pia-arachnoid at the graft site as well as the corresponding dorsal root ganglia were avulsed in other rats. Grafts in rats with

avulsed dorsal root ganglia contained a mean of 5,850 myelinated axons (s.e. = 510, $n = 6$); those in rats with intact spinal roots and ganglia contained a mean of 10,950 myelinated axons (s.e. = 1,660, $n = 5$). These results suggest that dorsal and ventral roots contributed to the innervation of the grafts but were not the sole source of axons. They do not exclude the possibility that the grafts were entirely innervated by axons originating from dorsal root ganglia and motor neurones¹².

A third group of experiments were carried out to identify the cellular origin of axons in the graft. In 15 rats, a segment of the spinal cord at least 10 mm long was removed and a sciatic nerve graft of equal length was inserted. The roots were left intact. Three to four months later the operative site was re-exposed and horseradish peroxidase (HRP) was injected into the spinal cord immediately rostral or caudal to the graft. After 2 more days, each animal was killed and labelled neurones were sought in a 10-mm segment of the non-injected spinal cord stump, immediately adjacent to the graft (Fig. 1). After injection rostral to the graft, the mean number of labelled neurones seen in the caudal spinal cord stump was 48 (0, 8, 28, 40, 169); after injection caudal to the graft, the mean number in the rostral stump was 6 (0, 0, 1, 6, 23). In five control animals, the graft was crushed with jeweller's forceps immediately before HRP injection and no HRP was seen 2 d later in spinal neurones on the other side of the graft. We conclude that neurones in the experimental groups were not labelled spuriously because of interstitial diffusion through the graft, flow in the cerebrospinal fluid or extravasation into the systemic vasculature. Labelling is thought to have resulted from incorporation of HRP into axon terminals at or near the spinal cord–graft junction and retrograde axonal transport across the graft to perikarya in the adjacent spinal cord. In segments below the graft, labelled

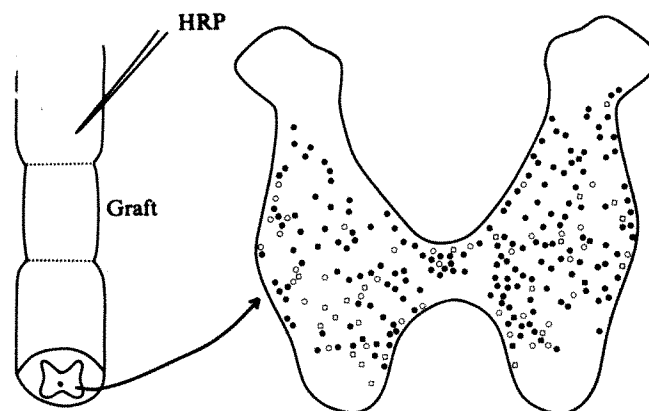


Fig. 1 Left, 2–4 months after the original operation, HRP (20% Sigma VI) was injected through a glass pipette into the spinal cord immediately rostral (or caudal) to the sciatic nerve graft. The injection of small quantities of HRP (0.5 μ l in 30 min) was designed to minimise the possibility of a false positive result due to the extravasation of enzyme and not to label every axon crossing the graft. Vaseline was spread extradurally and subdurally about the graft before injection, dry cotton batting was placed about the pipette during the injection and mineral oil was injected through the pipette as it was withdrawn. Two days later, the animal was perfused with glutaraldehyde. From a 10-mm segment of the spinal cord immediately across the graft from the injection site, specimens were removed and rinsed overnight in sucrose buffer. Approximately 300 sections, each 20 μ m thick, were cut on a freezing microtome, mounted, incubated with tetramethyl benzidine and hydrogen peroxide and counterstained with neutral red²⁴. Right, composite diagram illustrating the position of labelled nerve cell bodies in the spinal grey matter below the graft in four animals after HRP injection above the graft. Each animal is represented by a different symbol. Few neurones are labelled in the tips of the dorsal horns and in the lateral portion of the anterior horns. Because of the small amount of HRP injected and the short length of the spinal cord surveyed, it is assumed that more spinal neurones project into the graft than are shown here.

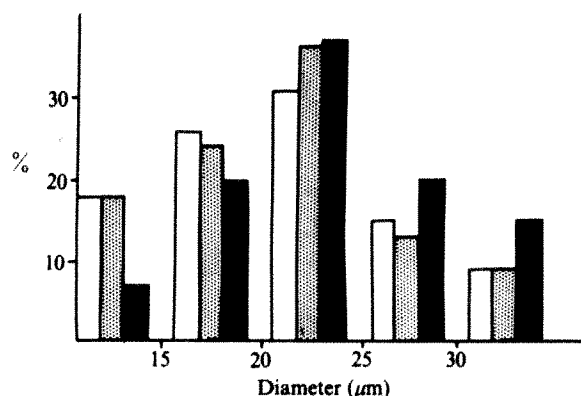


Fig. 2 Histogram showing the diameters as measured under the light microscope, of perikarya in the grey matter of the lower thoracic cord (excluding the dorsal part of the dorsal horn and lateral part of the ventral horn). White columns represent neurones in a normal rat; dotted columns represent unlabelled neurones below the graft; black columns represent labelled neurones below the graft. Labelled neurones have a larger mean diameter than either control group.

neurones were scattered through the central part of the grey matter (laminae 4, 5, 7, 8, 10)¹³ (Fig. 1). Both small and large nerve cells were labelled and the diameter of HRP-containing neurones was slightly greater than that of unlabelled neurones in the same regions of the spinal grey matter (Fig. 2). Some anterior horn cells above the graft and some dorsal root ganglia at the level of the graft and several segments below or above it also contained labelled cells but the ganglia were not surveyed systematically. The evidence demonstrates that some axons originating from neurones within the spinal cord grew a distance of approximately 10 mm into a PNS graft. We do not know whether such axons then re-entered the spinal cord and formed synapses. No functional improvement attributable to axonal regeneration was observed in any animal.

Mammalian central neurones with recognised capacity for axonal regrowth over a distance can be placed in two groups. In the first group, which includes somatic motor neurones and autonomic preganglionic neurones, the axons project outside the CNS. Intrinsic CNS neurones with regenerative ability have been found in the hypothalamus¹⁴ and brain stem^{15,16}. The latter neurones are cholinergic or monoaminergic and are thought to have thinly myelinated or unmyelinated axons¹⁷. The findings reported here indicate that there are other neurones in the rat spinal cord also capable of axonal elongation after injury. Some of the HRP-labelled perikarya probably represent autonomic preganglionic neurones about the central canal¹⁸ or in the intermediolateral columns, but most of them were found in areas of spinal grey matter normally occupied by neither autonomic nor somatic motor neurones. None is likely to have been monoaminergic because cell bodies containing catecholamines or serotonin have not been discovered in the spinal grey matter¹⁹. In short, the labelled cells do not belong to a class of neurones in which axonal regrowth has been described previously. It is unknown whether or not spinal axons with regenerative potentiality have any specific anatomical or biochemical property.

Spinal axons such as those which grew into a peripheral nerve graft in these experiments have repeatedly demonstrated only abortive sprouting after simple transection^{1,20}. This evidence that the regenerative response of similar axons differs in CNS and PNS neuroglia, together with other experimental observations^{10,21,22}, supports the hypothesis that Schwann cells are more conducive to axonal regeneration than central neuroglial cells²³. The regenerative potentiality of CNS neurones may be expressed only when their neuroglial environment is changed.

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Response to stress of mesocortico-frontal dopaminergic neurones in rats after long-term isolation

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Recent studies suggest that the mesocortico-frontal dopaminergic neurones which originate in the ventral tegmental area (VTA) have an inhibitory role in locomotor activity^{1,2}. They are also markedly activated under stress³⁻⁵. This effect was shown in rats and mice subjected to electric foot-shocks by measuring either the rate of decline of dopamine (DA) after α -methylparatyrosine treatment³ or the changes in dihydroxyphenylacetic acid (dopac) levels and the dopac/DA ratio^{4,6}. In rats, stress-induced activation of the dopaminergic neurones was prevented by benzodiazepines^{4,5}, and studies in BALB/c mice introduced for 2 min into an open field further established the role of dopaminergic neurones in emotional responses⁷. These observations led us to examine the effects of long-term isolation on the activity of the mesocortico-frontal dopaminergic neurones in rats, some of which were subjected to a stressful situation. Indeed, several groups have reported that long-term isolation in rodents induced behavioural disturbances such as increased motor activity^{8,9} and aggression^{9,10} and hyper-reactivity to a new environment or stressful stimuli^{10,11}. As measured by the changes in dopac levels or the dopac/DA ratio, we report here that the activity of the mesocortico-frontal dopaminergic neurones was reduced after isolation. This was not the case for the dopaminergic neurones projecting to the nucleus accumbens or the striatum, the rate of DA utilisation in these structures was even enhanced in isolated rats in which the activity of the mesocortico-frontal dopaminergic neurones was markedly reduced. Finally, we will show that a 3-min electric foot-shock session is more effective in enhancing dopac levels or the dopac/DA ratio in the frontal cortex of isolated than grouped rats.

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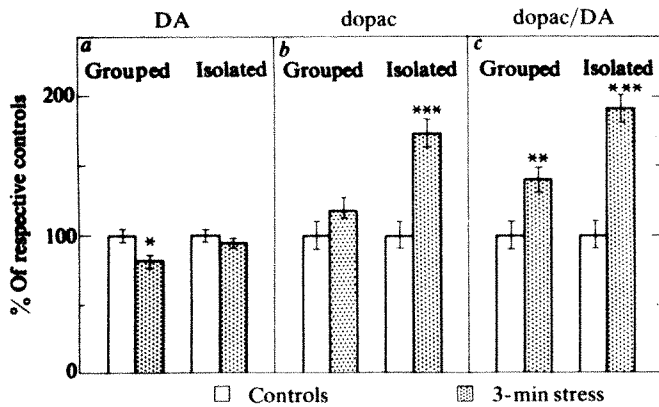


Fig. 1 Effect of stress on the rate of dopamine (DA) utilisation in the frontal cortex of grouped or isolated rats. Rats were isolated or kept in groups for 8 or 12 weeks and then individually exposed to a 3-min electric foot-shock stress. Other isolated or grouped rats were used as respective controls. In all cases, DA (a) and dopac (b) levels were estimated in the frontal cortex and the dopac/DA ratio (c) was calculated for each sample. Results are the mean of data from two independent experiments in which similar changes were observed. They are thus the mean \pm s.e.m. of data obtained with 20 samples (10 animals in each case) and are expressed in per cent of respective control values. The mean values of DA, dopac and dopac/DA ratios in grouped and isolated rats, respectively, are 1,150, 325, 0.29 and 1,250, 300, 0.26. DA and dopac levels being expressed in pg per mg of protein. \square , Controls; \blacksquare , 3-min stress. * $P < 0.05$; ** $P < 0.02$; *** $P < 0.001$ when compared with respective control values.

Male Charles River rats (180–200 g) were kept in a controlled environment (24 °C, 60% humidity, alternate cycles of 12 h light (0700–1900 h) and darkness) and received food and water *ad libitum*. Some of them were isolated in opaque cages (28 \times 18.5 \times 12 cm) for at least 8 weeks, others remained grouped (46 \times 26 \times 20 cm), (five animals per cage). The isolated rats could hear and smell their congeners, but were unable to see, touch or otherwise interact with them. Isolated and grouped rats were killed the same day between 1100 and 1200 h. Their brains were immediately frozen and cut in 500- μ m coronal slices from which tissue samples were punched out in both frontal cortices (11,300 and 10,800 μ m slices)⁴. In some experiments, samples were also taken bilaterally from the nucleus accumbens (9,800 and 9,300 μ m) and the medial part of the striatum⁴ (8,800 μ m) which is mainly innervated by nigral dopaminergic cells. After separation on alumina microcolumns, DA and dopac levels were estimated with a radioenzymatic method and the dopac/DA ratio was determined for each sample^{4,12}.

From the results of five independent experiments carried out during a 10-month period, the mean dopac/DA ratio was found to have decreased in frontal cortices of isolated rats (0.24 ± 0.010 , $n = 50$ samples, -30% , $P < 0.001$) when compared with that of grouped animals (0.34 ± 0.014 , $n = 50$). This effect, which was mainly due to a decrease in dopac levels (-19% , $P < 0.01$), suggests that isolation reduced the activity of the mesocortico-frontal dopaminergic neurones. However, the decreased dopac/DA ratio observed in isolated rats varied between experiments (-4% , -19% , -32% , -35% , -43%), the effect being significant in three cases. This seemed to be related to variations in the mean values of the dopac/DA ratio in the grouped rats (0.27–0.41); the ratio was remarkably constant from one experiment to another in isolated animals (0.23–0.26). Seasonal rhythms¹³ and/or seasonal changes in emotional states could be responsible for the variations observed in grouped rats. Prolonged isolation may attenuate these fluctuations.

In two of the five experiments, half of the rats isolated for 8 weeks were grouped for 4 additional weeks. In this case, their cortical dopac/DA ratio was significantly higher than that estimated in rats isolated for 12 weeks, but not different from that of rats continuously kept in groups (Table 1). Therefore, the reduced activity of the mesocortico-frontal DA neurones induced by isolation is a reversible process.

In two experiments we examined simultaneously the reactivity of the mesocortico-frontal DA neurones in grouped and isolated rats exposed for 3 min in individual cages to electric foot-shocks⁴. Confirming previous results, this stress reduced the levels of DA and increased the dopac/DA ratio in the frontal cortices of grouped rats⁴. The effect was more pronounced in isolated animals, the increase in the dopac/DA ratio being twice that observed in grouped rats and mainly due to an increase in dopac level ($+73\%$) (Fig. 1). Because the absolute levels of dopac and the dopac/DA ratio were higher in isolated (519 ± 45 pg per mg protein, 0.50 ± 0.04 , $P < 0.05$) than in grouped (383 ± 36 pg per mg protein, 0.40 ± 0.03) rats, the enhanced reactivity of the DA neurones seen under stress in isolated animals cannot be attributed solely to their initial lower state of activity. The biochemical changes observed in isolated rats, which were identical to those previously seen in grouped animals exposed to a longer stress session (20 min)⁴ suggest that the intense activation of the DA neurones enhanced not only the rate of DA utilisation but also its synthesis. Behavioural differences were also observed: the grouped rats jumped during electric shocks and attempted to escape, whereas the isolated rats exhibited stereotypic and freezing behaviours during and between shocks, respectively. The grouped rats were highly reactive at the end of the 3-min stress whereas the isolated animals seemed exhausted.

Finally, in three of the five experiments we also compared the rate of DA utilisation in the nucleus accumbens and the striatum of isolated and grouped rats. The mean results show a slight but not significant enhanced utilisation of DA in these structures in isolated rats. This finding is in contrast with the effect observed in the frontal cortex. Moreover, in one experiment in which a pronounced reduction of the dopac/DA ratio was seen (-43%), significant opposite effects were obtained in the nucleus accumbens ($+36\%$) and striatum ($+33\%$) (Fig. 2).

Changes in monoamine metabolism in the whole brain of isolated rats or mice have already been reported. Despite some discrepancies, most authors found that the turnover rate of serotonin, noradrenaline and DA were reduced in isolated rats^{8–11}. In one study, DA turnover was decreased in the amygdala but was slightly increased in the olfactory tubercles and remained unchanged in the striatum and the nucleus accumbens¹⁴.

Our results extend previous findings and demonstrate that long-term deprivation of social interaction in rats reduced and

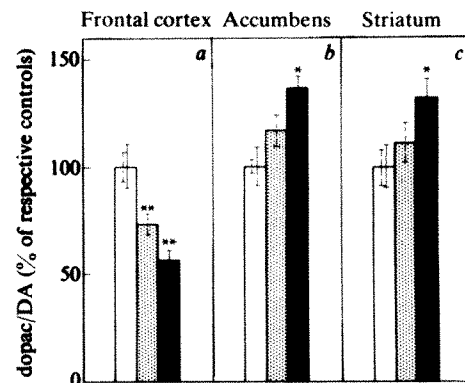


Fig. 2 Effects of isolation on the dopac/DA ratio in the frontal cortex, nucleus accumbens and striatum. Rats were isolated or kept in groups for 8 or 12 weeks and the dopac/DA ratio was estimated bilaterally in areas of the frontal cortex (a), nucleus accumbens (b) and striatum (c). For each structure, the results are the mean \pm s.e.m. of 30 samples (15 rats) and 10 samples (5 rats), respectively. They are expressed in per cent of corresponding values: 0.33, 0.15, 0.09 (30 samples) and 0.41, 0.18, 0.12 (10 samples) for the frontal cortex, nucleus accumbens and striatum, respectively. \square , Grouped; \blacksquare , isolated, mean of three independent experiments; \blacksquare , isolated, values of one of three experiments in which the maximal decrease in the cortical dopac/DA ratio was observed.

* $P < 0.02$, ** $P < 0.001$ when compared with respective control values.

Table 1 Reversibility of the effects of long-term isolation on the dopac/DA ratio and dopac levels in the rat frontal cortex

	dopac/DA	dopac (pg per mg protein)
Grouped (12 weeks)	0.40 ± 0.024	430 ± 35
Isolated (12 weeks)	0.24 ± 0.010	309 ± 19
	(-40%)*	(-28%)†
Isolated (8 weeks) then grouped (4 weeks)	0.31 ± 0.017	401 ± 24
	(+29%)‡	(+30%)‡

Rats were isolated or kept in groups (five per cage) for 12 weeks. Other animals were first isolated for 8 weeks and then grouped for 4 weeks. DA and dopac levels were estimated bilaterally in the frontal cortex and the dopac/DA ratio was calculated for each sample. Results are the mean of data obtained in two independent experiments in which similar changes were observed. Thus, values of the dopac/DA levels are the mean ± s.e.m. of data obtained with 20 samples (10 animals) in each case.

* $P < 0.001$; † $P < 0.01$ when compared with values obtained with grouped animals (Student's *t*-test). ‡ $P < 0.01$ when compared with values obtained with rats isolated for 12 weeks.

maintained at a low level the activity of their mesocortico-frontal dopaminergic neurones. Indeed, the cortical dopac/DA ratio was constant during a 10-month period and was lower than that observed in grouped animals. Furthermore, isolation enhanced the reactivity of the mesocortico-frontal dopaminergic neurones to stress, as a 3-min session of electric foot-shocks was more effective in enhancing the cortical dopac levels and dopac/DA ratio in isolated than in grouped animals. This suggests that under stress the transmission of messages responsible for the activation of the mesocortico-frontal dopaminergic neurones is facilitated by previous isolation.

In recent studies, we showed that mesocortico-frontal dopaminergic neurone activity was controlled by interneuronal regulations which differed from those of other VTA dopaminergic cells^{15,16}. The present results confirm these findings because, in contrast to that observed for the mesocortico-frontal dopaminergic neurones, the activity of the dopaminergic neurones innervating the nucleus accumbens was not reduced in isolated rats. In fact, the opposite occurred in both the nucleus accumbens and the striatum, when the cortical dopac/DA ratio was reduced to a large extent in isolated rats. These results agree with Carter and Pycock's recent observations suggesting that the mesocortico-frontal dopaminergic neurones exert an inhibitory role on the activity of dopaminergic neurones innervating subcortical structures². Indeed, these authors have shown that the specific destruction of fronto-cortical dopaminergic terminals in the rat not only enhanced the turnover of DA in the nucleus accumbens and striatum but also increased the locomotor activity and stereotypic effect of amphetamine².

We already know that the mesocortico-frontal dopaminergic neurones are involved in the control of locomotor activity^{1,2}, emotional behaviour^{4,17} and cognitive processes^{18,19}. Therefore, the prolonged reduced activity of the mesocortico-frontal dopaminergic neurones in isolated rats as well as their higher reactivity under stress could be responsible for the enhanced locomotor activity and hyperactivity seen in these animals.

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Cycloheximide-sensitive synthesis of substance P by isolated dorsal root ganglia

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Substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂) may be used as a neurotransmitter by certain primary afferent neurones^{1,2}, particularly those carrying pain impulses³⁻⁷. Substance P-like immunoreactivity has been localised to the cell bodies of one population of dorsal root ganglion neurones by immunocytochemistry⁸. It is contained in vesicles^{9,10} in the central terminals of these neurones⁸, and has also been demonstrated in the peripheral terminals^{11,12}. As axons and terminals have very little capacity for peptide biosynthesis¹³, it is possible that substance P is synthesised and packaged in the perikaryon and transported to the terminals by an axoplasmic transport process. Consistent with this is the finding that substance P accumulates proximal to a ligature placed on the dorsal root¹⁴. There has, however, been no direct demonstration of the biosynthesis of substance P in the nervous system. We report here that rat dorsal root ganglia incorporate ³⁵S-methionine into substance P, characterised as authentic by immunoprecipitation followed by HPLC. There is a delay of 1-2 h between addition of label and its incorporation into substance P. Synthesis is blocked by cycloheximide suggesting that, in dorsal root ganglia, substance P is synthesised by a conventional ribosomal process. Synthesis of substance P is reduced by some 90% in ganglia from rats treated neonatally with capsaicin, a drug which is thought to destroy a population of primary afferent neurones¹⁵.

The anti-substance P serum was raised in rabbits using synthetic substance P (UCB Bioproducts) conjugated to succinylated thyroglobulin by the carbodiimide method. As expected with this coupling reagent, which reacts with the amino groups on residues 1 and 3 of substance P, the antiserum was directed to the C-terminal region of the peptide. The affinity of a number of substance P analogues for the antiserum was tested by radioimmunoassay using ¹²⁵I-Tyr⁸ substance P as tracer. The affinities of fragment 4-11, the substance P analogue H-Lys-Phe-Tyr-Gly-Leu-Met-NH₂ (CGP 15 898) and of substance P-free acid, which differs from substance P only in that it lacks the C-terminal carboxamide group, were respectively 2-, 10- and 4,000-fold less than that of the parent peptide.

Rat lumbar dorsal root ganglia were incubated with ³⁵S-methionine, homogenised, centrifuged and the radiolabelled peptides in the supernatant immunoprecipitated with anti-substance P serum. The immunoprecipitates were washed, redissolved by boiling in HPLC buffer and the radiolabelled peptides separated by reverse-phase HPLC (see legend to Fig. 1 for experimental details). In initial experiments designed to establish the specificity of the immunoprecipitation, washed immunoprecipitates were incubated overnight with a large excess (50 µg) of substance P in buffer A (see legend to Fig. 1). After centrifugation the supernatant was diluted with 1 ml buffer B and the labelled peptides separated by HPLC in the usual way. Elution by displacement with substance P and by

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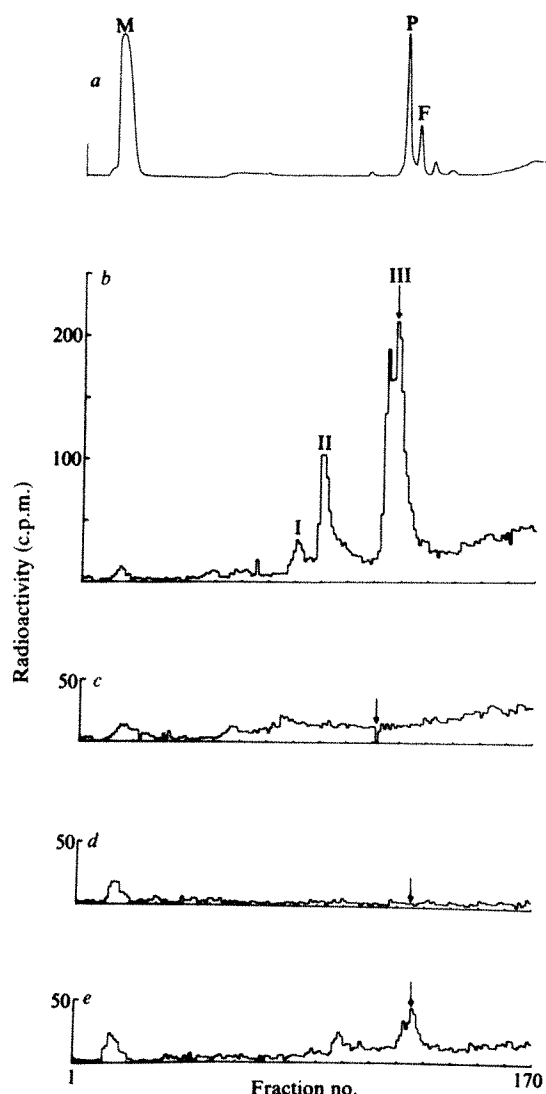


Fig. 1 HPLC of immunoprecipitates from rat dorsal root ganglia incubated with ^{35}S -methionine for 9 h. *a*, Standards detected by UV absorption. M, methionine; P, substance P; F, substance P fragment 4–11. *b–e*, distribution of radioactivity. Arrow denotes position of carrier substance P: *b*, precipitated with immune serum; *c*, same but precipitated with pre-immune serum; *d*, as *b* but 0.1 mM cycloheximide included in incubation medium; *e*, dorsal root ganglia from rat which had been treated neonatally with capsaicin. Rats were perfused with ice-cold Krebs–bicarbonate. Dorsal root ganglia L4–6 were dissected out, placed in 0.5 ml of incubation medium containing (mM) NaCl, 118; KCl, 4.5; CaCl_2 , 2.5; MgCl_2 , 1.15; HEPES (pH 7.5), 15; glucose 28 and 100 μCi ^{35}S -labelled L-methionine (Radiochemical Centre, 710 Ci mmol $^{-1}$), incubated with shaking at 37 °C under O_2 for 9 h, washed, held in 200 μl 2M acetic acid in a boiling waterbath for 10 min and homogenised. In experiments with cycloheximide ganglia were pre-incubated for 1 h with 0.1 mM cycloheximide, ^{35}S -methionine was then added and incubation was allowed to proceed for a further 9 h in the continued presence of cycloheximide. The homogenate was centrifuged and the supernatant lyophilised. The lyophilised samples were taken up in 250 μl buffer A (50 mM barbital buffer, pH 8.6 containing 1 mM EDTA, 0.5 mg ml $^{-1}$ L-methionine and 0.1% Triton X-100) and centrifuged. Aliquots (110 μl) of the supernatant were incubated for 16 h on ice with 20 μl buffer A (to which had been added 20 mg ml $^{-1}$ bovine serum albumin and 50 μg ml $^{-1}$ poly-lysine) and 10 μl anti-substance P serum or pre-immune serum. 14 μl goat anti-rabbit IgG serum (Miles) and 26 μl buffer A were then added and immunoprecipitates were allowed to form for a further 4 h at 4 °C. All sera were pretreated with phenylmethyl sulphonyl fluoride and iodoacetamide to inhibit protease activity 27 . Immunoprecipitates were washed three times with 1 ml buffer A then redissolved in 1 ml buffer B (0.1 M NaH_2PO_4 , 0.1 M H_3PO_4 , pH 2.1) containing 50 μg substance P, held in a boiling waterbath for 10 min and injected on to a 250 \times 4.6 mm column of Partisil 10 ODS using a 2 ml sample loop. After a loading phase of 3 min in buffer B the peptides were eluted with a gradient of acetonitrile in buffer B essentially as described by O'Hare and Nice 20 and terminating when the acetonitrile concentration reached 60% (v/v). Carrier substance P was detected by UV absorption at 225 nm and 10-drop fractions were collected for liquid scintillation counting in a Triton X-100 toluene-based scintillation fluid at 80% efficiency.

direct dissolution in HPLC buffer gave similar results, so, as the latter procedure was simpler, it was used routinely.

The ^{35}S -labelled material precipitated by anti-substance P serum separated as three well-defined peaks, one of which (peak III) coincided with authentic carrier substance P (Fig. 1*b*). None of these peaks corresponded with substance P sulfoxide which elutes at a point intermediate between peaks II and III. There was a lag phase before ^{35}S -methionine became incorporated into the substance P peak; no incorporation was detectable after 1 h but it became apparent at 2 h and continued to rise at 3 h and 8 h (Fig. 2). Incorporation of label into peak II followed a similar time course, whereas the radioactivity incorporated into peak I had reached a maximum at 2 h and thereafter remained essentially constant. There was no radioactivity in any of the three peaks when the anti-substance P serum in the immunoprecipitation step was replaced by serum taken from the same animal before immunisation (Fig. 1*c*). Incorporation of radiolabel into substance P was completely blocked by inclusion of 0.1 mM cycloheximide in the incubation medium (Fig. 1*d*).

In ganglia from 10-week-old rats which had been given 0.7 mg capsaicin on the second day of life 15 , incorporation into substance P (peak III) and into peaks I and II was respectively 12, 8 and 14% of that in normal animals (Fig. 1*e*). Neonatally-administered capsaicin causes degeneration of a population of small-diameter chemosensitive neurones 15,16 which are thought to use substance P as a neurotransmitter 3 . Animals treated in this way have severely reduced levels of substance P in the dorsal horn of the spinal cord 17 and in their dorsal root ganglia (unpublished observation). Morphometric studies of the ganglia and dorsal roots of these animals show that the population of small dark cells and of non-myelinated fibres is severely depleted while the population of myelinated fibres is slightly depleted 18 .

The substance P peak and also peak II were re-chromatographed on a 58 \times 1 cm column of Sephadex G-50 in phenol: acetic acid: water 1:1:1, containing 0.5% mercaptoethanol 19 . The substance P peak eluted as a single peak on Sephadex G-50 which coincided with ^{125}I -Tyr 8 substance P, confirming its identity with authentic substance P. Peak II eluted later than ^{125}I -Tyr 8 substance P on Sephadex G-50, suggesting that it is smaller than substance P. To test the possibility that peak II could have been formed artefactually from ^{35}S -substance P during extraction and chromatography, we added ^{125}I -Tyr 8 substance P to a ganglion homogenate and carried it through the complete procedure. The ^{125}I -Tyr 8 substance P treated in this way gave no extra peaks and so it seems likely that both peaks I and II represent peptides synthesised in the tissue. If peak II is a fragment of substance P it must be a C-terminal fragment as it contains ^{35}S -methionine. The retention time of peptides on reverse-phase supports has been shown to be broadly correlated with their hydrophobic amino acid content 20 . Thus fragments of substance P containing phenylalanine residues at both positions 7 and 8 may be expected to have similar or longer retention times than substance P. For example, fragment 4–11 is retained longer than substance P (Fig. 1*a*). As peak II elutes before substance P it could be a fragment in which one or both the phenylalanine residues has been lost. We are currently attempting to elucidate its structure.

The complete inhibition of substance P biosynthesis by cycloheximide (Fig. 1*d*) suggests that the peptide is synthesised by a conventional ribosomal mechanism. Substance P is released from nerve terminals 7,21,22 and so it is to be expected that, in common with other exported peptides 23,24 , substance P is synthesised on membrane-bound ribosomes. Consistent with this is the observation that the perikarya of the small dark neurones, in which substance P-like immunoreactivity is localised 8 and which are destroyed by capsaicin 15 , are replete with rough endoplasmic reticulum 25 . As it is thought that all peptides synthesised on membrane-bound ribosomes must be at least 40–60 residues in length and bear a hydrophobic N-terminal signal sequence 26 one would expect the undecapeptide substance P to be formed from a larger precursor. The latent period of 2 h between the addition of ^{35}S -methionine and the

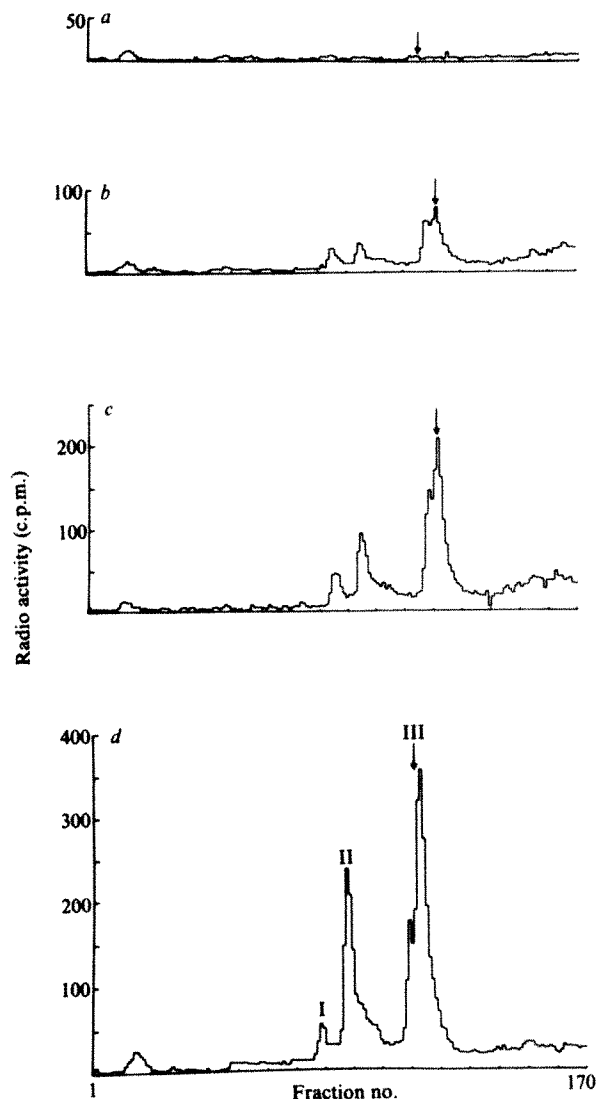


Fig. 2 HPLC of immunoprecipitates from rat dorsal root ganglia incubated with ^{35}S -methionine for 1, 2, 3 and 8 h. Method as in legend to Fig. 1 except that the entire supernatant from each homogenate was reacted with anti-substance P serum. Arrows denote position of carrier substance P.

appearance of the label in substance P (Fig. 2) may represent the time necessary for the processing of such a precursor. Note that the radioactivity incorporated into peak I had reached a maximum at 2 h and thereafter remained constant while incorporation into substance P continued to rise (Fig. 2). This is the behaviour expected of a precursor. Unfortunately the radioactivity incorporated into peak I in these experiments was insufficient to allow us to determine its size by subsequent chromatography on Sephadex G-50.

The present demonstration that isolated rat dorsal root ganglia are able to incorporate ^{35}S -methionine into substance P provides a system in which the synthesis and packaging of neurotransmitter substance P may be examined in detail. Using this system we have obtained evidence that substance P synthesis in dorsal root ganglia occurs by a ribosomal mechanism and that capsaicin may be a useful tool for the selective destruction of neurones responsible for this biosynthesis. We are continuing to investigate the possibility that substance P may be synthesised from larger molecular weight precursors.

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Enkephalin-, VIP- and substance P-like immunoreactivity in the carotid body

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The carotid body type I cell contains amines^{1–3} and has features, both morphological and cytochemical, which indicate that it may also produce a peptide^{4–6}. Many regulatory peptides are now known to be present in both central and peripheral tissues⁷. In the periphery these neuropeptides occur in both classical endocrine (APUD) cells and the neurones of the autonomic nervous system⁸. We have now investigated the possible presence of neuropeptides in the cat carotid body using both immunocytochemistry and radioimmunoassay. Met- and Leu-enkephalin-like material occurred in considerable quantities in carotid body extracts and enkephalin-like immunoreactivity was localised in type I cells. Both vasoactive intestinal polypeptide (VIP)- and substance P-like immunoreactivity was also present but was localised in nerve fibres distributed throughout the organ. These active neuropeptides are widely distributed in mammalian tissues, forming a diffuse regulatory system which now seems to include the carotid body.

Antisera to Met-, Leu-enkephalin, VIP and substance P were raised in rabbits and characterised for both radioimmunoassay (Table 1) and immunocytochemistry (Table 2). The carotid bodies and bifurcation of the common carotid arteries were removed from adult cats ($n = 25$) under pentobarbitone anaesthesia.

For immunocytochemistry, two methods of fixation were used. (1) The whole bifurcation, including the carotid body, was frozen in melting freon (Arcton), freeze dried, fixed in benzoquinone vapour for 3 h at 60 °C and embedded in paraffin wax⁹.

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Table 1 Characterisation of antisera for enkephalin, VIP and substance P radioimmunoassays

Antiserum to	Carrier and coupler	Label	Sensitivity	Titre	Characterisation
Met-enkephalin	Bovine serum albumin (BSA) by glutaraldehyde	³ H-Met enkephalin	20–0.025 pmol <0.2% cross reactivity with Leu-enkephalin	1:1,500	C-terminal
VIP	BSA by carbodiimide	¹²⁵ I Chloramine-T method Pure porcine VIP	1 fmol per assay tube	1:320,000	C-terminal
Substance P	BSA or α -globulin by glutaraldehyde	¹²⁵ I Chloramine-T method Tyr ⁸ substance P	0.3 fmol per assay tube	1:8,000	C-terminal

(2) Tissue for liquid fixation was immersed in a 0.4% solution of benzoquinone in 0.01 M phosphate-buffered normal saline (pH 7.1–7.4) for up to 30 min at 4 °C (ref. 10). It was also rinsed several times in phosphate-buffered saline containing 7% sucrose and 0.01% sodium azide and cryostat blocks were prepared. Dewaxed and cryostat sections were stained by either the indirect immunofluorescence method¹¹ or the peroxidase–antiperoxidase (PAP) technique¹². Sections were incubated for 16–20 h at 4 °C, with antisera diluted in phosphate-buffered saline.

For radioimmunoassay the two carotid bodies from each cat were pooled and extracted twice with 500 μ l of 0.1 M acetic acid or with 500 μ l of water and 500 μ l of 0.1 M formic acid. The extracts were assayed in serial dilutions for VIP, substance P- and enkephalin-like immunoreactivity. The nature of the enkephalin-like material was examined using both column chromatography (Fig. 3) and thin layer chromatography¹³ (TLC). The carotid bodies were composed of clusters of cells separated by connective tissue and a rich vascular network. Large vessels, nerves and occasional ganglion cells were observed in the periphery of the organ.

Following incubation with either Met- or Leu-enkephalin antisera, immunoreactive cells were observed in the carotid body (Fig. 1). These cells were arranged in groups corresponding to the type I cell clusters. Immunostaining of serial sections revealed that the antisera stained the same groups of cells. No immunoreactive nerve fibres were seen in sections incubated with enkephalin antisera and type I cells were not stained by either VIP or substance P antisera.

An extensive network of varicose nerve fibres containing VIP-like immunoreactivity was observed (Fig. 2), associated principally with blood vessels and enkephalin immunoreactive type I cell clusters. VIP-like material also occurred in nerve fibres around the carotid arteries and in ganglion cells in the periphery of the carotid body. Fine nerve fibres immunostained with substance P antiserum were also distributed throughout the organ. The staining of each antiserum was completely abolished by preabsorption of the antiserum with its respective antigen (Table 2).

Significant amounts of VIP- (71.7 ± 18.4 pmol per g (\pm s.e.m.) $n = 5$), substance P- (54.6 ± 16.8 pmol per g) and enkephalin-like immunoreactivity (982.6 ± 203.8 pmol per g) were found in

the carotid body extracts. Examination of the extracts by TLC revealed that both Met- and Leu-enkephalin-like material was present, with between three and four times more Met- than Leu-enkephalin. On a BioGel column the enkephalin-like material eluted at the same position as ³H-labelled, synthetic Met-enkephalin (Fig. 3). In addition, a higher molecular weight immunoreactivity was detected which eluted with the void volume.

Our results reveal that, as in other organs⁸, the carotid body possesses an extensive network of nerve fibres containing substance P- and VIP-like immunoreactivity. The origin of these fibres is not entirely clear. Those in which VIP-like immunoreactivity occurs may, at least in part, arise locally, for scattered immunoreactive ganglion cells were seen in the periphery of the organ, whereas those containing substance P-like material may originate outside the carotid body. Substance P is reported to affect chemosensory activity in the cat carotid body¹⁴. However, the role of these peptides in this organ is unknown.

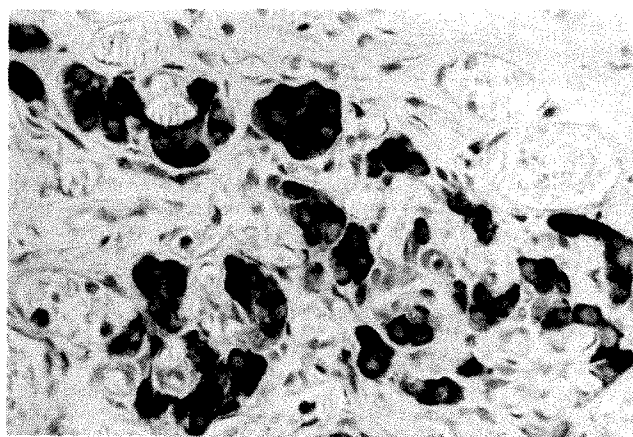


Fig. 1 Enkephalin-like immunoreactive type I cells in a 3- μ m thick section from a cat carotid body fixed in benzoquinone vapour ($\times 300$). Immunostained by the peroxidase–antiperoxidase technique with antiserum to Met-enkephalin (1:1,600 dilution).

Table 2 Characterisation of antisera for immunocytochemistry

Antiserum to:	REF	IF	Titre	Absorption (0.1–40 nmol per ml of diluted antiserum)					
				M-Enk	L-Enk	β -End	β -LPH	VIP	Sub P
Met-enkephalin	(497)	1:400	1:1,600	–(5)	–(40)	<(40)	<(40)	+	+
Met-enkephalin	(579)	1:200	1:1,000	–(5)	–(1)	<(20)	+	+	+
Leu-enkephalin	(493)	1:200	1:800	–(40)	–(5)	<(40)	+	+	+
Leu-enkephalin	(578)	1:200	1:1,000	–(5)	–(1)	–(20)	–(40)	+	+
VIP	(324)	1:300	1:2,000	+	+	+	+	–(0.1)	+
Substance P	(479)	1:500	1:4,000	+	+	+	+	+	–(5)

IF, Immunofluorescence technique¹¹; PAP, peroxidase–antiperoxidase technique¹²; REF, antiserum reference number. Controls included the application of antiserum preabsorbed with Met-enkephalin (M-Enk), Leu-enkephalin (L-Enk), β -endorphin (β -End), β -lipotropin (β -LPH), VIP or substance P (Sub P). +, No change in the intensity of the immunostaining; – (nmol ml^{–1}), minimum amount of peptide required to abolish the immunostaining; < (nmol ml^{–1}), amount of peptide required to reduce significantly the intensity of the immunostaining.

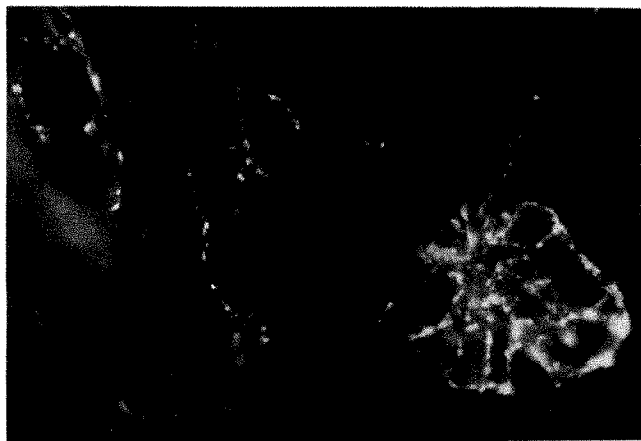


Fig. 2 Varicose nerve fibres containing VIP-like immunoreactivity, associated with a cluster of type I cells ($\times 415$). A 10- μ m-thick cryostat section incubated with antiserum to VIP (1:300 dilution) and immunostained by the indirect immunofluorescence technique.

Several peptides are known to be stored with amines in a single cell^{6,15-17} or neurone¹⁸⁻²¹, and it has recently been reported that adrenal chromaffin cells^{22,23} and their tumours^{22,24} contain enkephalin-like peptides in addition to catecholamines. The finding of enkephalin-like material in carotid body type I cells provides another example of the co-existence of amines and peptides in the same cell.

Both β -endorphin and β -lipotropin contain the molecular sequence of Met-enkephalin^{25,26}; thus, the partial abolition of the immunostaining by these peptides (Table 2), particularly that of Met-enkephalin, is not unexpected and may indicate the presence of a larger precursor molecule. The larger molecular weight, immunoreactive substance found in the extracts may be similar to that previously reported to occur in sympathetic ganglia²⁷.

It is well known that opiates depress respiration²⁸, but little information is available regarding their role in the carotid body. Opiates affect the release of central and peripheral neurotransmitters²⁹⁻³⁴ and the catecholamine content of adrenal medullary cells^{32,35}. It has been proposed that the two predominant catecholamines in the carotid body, dopamine and noradrenaline, modulate chemoreceptor discharge³⁶⁻³⁸. Thus, the association of amines and peptides may be of importance for the chemoreceptor properties of the carotid body.

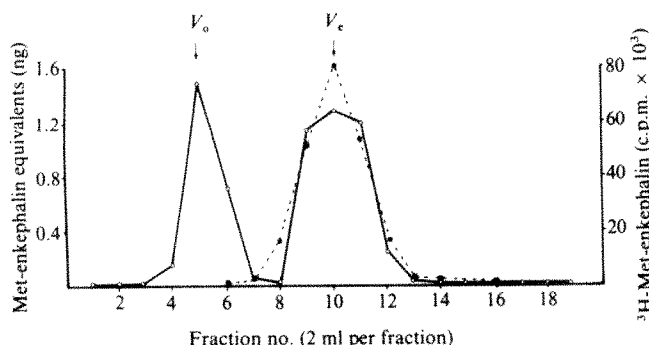


Fig. 3 Gel permeation chromatography profiles of a cat carotid body extract (○) and ³H-labelled synthetic Met-enkephalin (■). Pooled formic acid and water extracts (1 ml) were applied to a BioGel P2 column (10 cm \times 1 cm) and eluted with a 100 mM sodium phosphate buffer (pH 7.4) containing 50 μ M bacitracin, at a flow rate of 1 ml per 3 min 200,000 c.p.m. ³H-Met-enkephalin was applied to the same column on a separate run, and detected in the effluent fractions by scintillation counting. V₀, Void volume; V_c, enkephalin.

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Selective inhibition of prostaglandin production in inflammatory exudates and gastric mucosa

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A major clinical problem encountered in the use of non-steroid anti-inflammatory drugs has been the high incidence of gastrointestinal irritation. The mechanisms underlying damage to the gastric mucosa evoked by these drugs are complex. The normal resistance of the gastric mucosa to the back-diffusion of gastric acid from the lumen into the mucosal tissue can be disrupted by topical administration of aspirin as well as several irritants such as ethanol, bile salts and detergents; this results in gastric mucosal damage¹. Such an action, however, cannot be the sole mechanism because many non-steroid anti-inflammatory drugs cause gastrointestinal damage when administered parenterally^{2,3}. It has been proposed that a reduction in prostaglandin biosynthesis by aspirin-like drugs⁴ in the gastric mucosa leads to a fall in local blood flow which, in turn, gives rise to areas of focal ischaemia ultimately developing into erosions⁵. However, a combination of the direct topical irritant actions and inhibition of prostaglandin formation can lead to a marked potentiation of gastric damage³. Such a situation is likely to be encountered in the clinical use of these compounds following

oral administration. We have investigated the relationship between gastric damage and inhibition of cyclooxygenase in the gastric mucosa following oral administration of anti-inflammatory compounds. For these studies, we have measured the *ex vivo* formation of prostacyclin (PGI_2), the major cyclooxygenase product in the rat gastric mucosa which, like other prostaglandins such as PGE_2 , has gastro-protective actions⁶. In the inflammatory exudate induced by carrageenin, PGE_2 predominates and has pro-inflammatory actions⁷. We have, therefore, investigated whether selective inhibition of prostaglandin production, assayed as PGE_2 -like activity, in the inflammatory exudate can be achieved *in vivo*. We report here that it can.

Previous studies have shown that cyclooxygenase inhibition *in vivo* prevents subsequent prostacyclin generation by the rat gastric mucosa *ex vivo*⁸. Strips of gastric mucosa (0.2–0.4 g) freed from underlying muscle were rapidly washed, chopped and incubated in buffer (50 mM Tris, pH 8.4, 1 ml) by vortex mixing (1 min, 22 °C). After centrifugation in a fixed-speed Eppendorf bench centrifuge (15 s at 9,000g) the supernatant was immediately tested for its ability to inhibit human platelet aggregation. This prostacyclin-like activity, which was characterised as described before⁸ and by the use of an antiserum which binds and inactivates prostacyclin⁹, was assayed against authentic prostacyclin.

Inflammatory exudates in male rats (200 g body weight) were induced and collected by the subcutaneous implantation of polyester sponges impregnated with carrageenin (20 mg ml⁻¹ in sterile saline). After 24 h, the sponges were removed, immersed in heparinised saline (5 ml) and squeezed until dry. The pros-

taglandin-like activity in acid-lipid extracts of these exudates was determined by bioassay on the rat superfused stomach strip, treated with a mixture of antagonists, and was expressed as PGE_2 equivalents¹⁰.

Erosion formation in the gastric mucosa was determined as described previously³. Food was removed from the individually caged rats immediately after initial drug administration. The animals were killed after 24 h and the incidence and severity of the erosions, which formed only in the secretory mucosa, was assessed. The compounds were suspended in Celacol solution (0.25% w/v carboxymethylcellulose) and the suspension adjusted to pH 5–6. Drugs were administered orally (0.1 ml per 100 g body weight), three times during the 24 h period, at the time of sponge implantation, 6 h later and again 3 h before removal of sponge or assessment of mucosal erosion formation and prostacyclin production.

The doses of the compounds given (see Fig. 1 legend) are all in the range causing 50–70% inhibition of inflammation, as determined by reduction in the oedema induced 3 h after the subplantar injection of carrageenin in the hind paw of rats^{11,12}. The dose causing 50% reduction of rat paw oedema (ED_{50}) was (in mg per kg) for aspirin, 130; sodium salicylate, 140; indomethacin, 2.7; flurbiprofen 0.4; naproxen, 5. The recently described dual inhibitor of cyclooxygenase and lipoxygenase, BW755C (3-amino-1-([*m*-(trifluoromethyl)-phenyl]-2-pyrazoline)¹² was administered in doses greater than those required for the anti-inflammatory actions (ED_{50} 11 mg per kg).

The clinically used compounds all reduced cyclooxygenase products in both the sponge and the gastric mucosa in these anti-inflammatory doses (Fig. 1B). Both aspirin and flurbiprofen seemed more active on cyclooxygenase in the gastric mucosa than in the inflammatory exudate. In contrast, sodium salicylate and BW755C, in doses causing a significant reduction in prostaglandins in the sponge, failed to inhibit mucosal cyclooxygenase activity. In further studies, the compounds were administered by a single oral dose and prostacyclin generation by the gastric mucosa was determined 3 h later. Sodium salicylate (200 mg per kg) and BW755C (100 mg per kg) again failed to prevent mucosal cyclooxygenase activity, whereas aspirin (50–200 mg per kg) and indomethacin (1.25–10 mg per kg) were potent inhibitors.

The activity of these compounds on gastric mucosal cyclooxygenase activity 3 h after subcutaneous injection was also investigated. Sodium salicylate (50–400 mg per kg) and BW755C (25–150 mg per kg) caused no significant inhibition of *ex vivo* prostacyclin formation, whereas aspirin (50–200 mg per kg), indomethacin (1.25–10 mg per kg), naproxen (2.5–20 mg per kg) and flurbiprofen (0.1–1 mg per kg) all caused dose-dependent reductions in prostacyclin production. This indicates that the potent inhibition of mucosal cyclooxygenase by these latter compounds is not simply a consequence of selective accumulation in the gastric mucosal tissue following administration of local high concentrations of these agents.

To investigate whether the failure to prevent prostacyclin formation was due to washout or dilution of the drug from the mucosa during the incubation procedures, gastric mucosal tissue from rats pretreated with sodium salicylate (200 mg per kg) was washed, chopped and incubated in buffer solutions containing sodium salicylate (10–100 $\mu\text{g ml}^{-1}$). Again, no inhibition of prostacyclin production could be detected. It has been suggested that the inhibition of prostacyclin production in rat aortic rings *in vitro* by indomethacin pretreatment can be reversed by washing and incubation of the tissue¹³. However, with our procedure and using gastric mucosal tissue, we have shown that pretreatment of rats with aspirin, indomethacin, naproxen and flurbiprofen always led to a potent inhibition of prostacyclin production *ex vivo*, suggesting little tissue washout or irreversible cyclooxygenase inhibition with these drugs.

The formation of gastric erosions following oral administration of those agents in anti-inflammatory doses, three times over 24 h, is shown in Fig. 1A, expressed in terms of an erosion

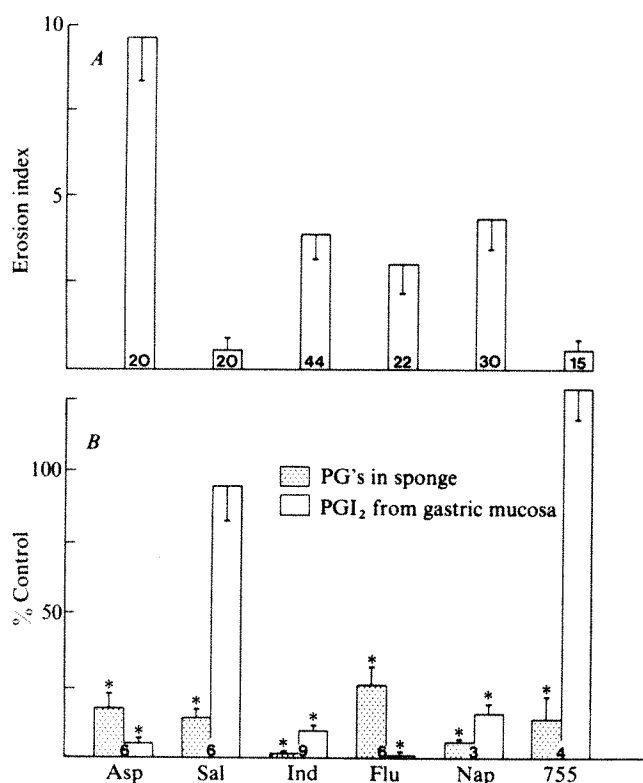


Fig. 1 Effect of oral administration of non-steroid anti-inflammatory agents on the induction of gastric erosions in rats (A), and prostaglandin E_2 -like activity in the sponge inflammatory exudate and the formation of prostacyclin (PGI_2) by the gastric mucosa *ex vivo* (B). The results are expressed as the mean \pm s.e.m. of *n* values. The changes in prostaglandin levels are given as % change compared with control levels, where an asterisk indicates significance ($P < 0.01$). The degree of gastric damage is expressed in terms of an erosion index. Drugs were administered orally three times over 24 h in the following doses (mg per kg): aspirin (200); sodium salicylate (200); indomethacin (4); flurbiprofen (0.5); naproxen (5); BW755c (100).

index. Indomethacin, flurbiprofen and naproxen induced a comparable degree of gastric erosion formation which paralleled their efficacy in inhibiting mucosal prostacyclin production in the doses used. Aspirin, however, was more active in inducing gastric damage in doses causing a similar mucosal cyclooxygenase inhibition to the other compounds. This may reflect the synergistic interaction³ between the topical irritation known to occur with aspirin^{1,2} and the inhibition of mucosal cyclooxygenase.

Interestingly, sodium salicylate, which failed to inhibit mucosal prostacyclin production in anti-inflammatory doses, caused little gastric damage. This finding is similar to observations in man that sodium salicylate produced far less gastrointestinal irritation than aspirin, as determined by faecal blood loss^{2,14}. Thus, topical irritation alone may not lead to pronounced gastric bleeding and erosion formation. Previously, it has been shown that systemically administered salicylic acid failed to prevent thromboxane A₂ formation in rat platelet-rich plasma, a situation in which washout of an active drug could not occur¹⁵. Furthermore, in our own studies on prostacyclin production *ex vivo* by rat isolated aortic rings, prior systemic administration of sodium salicylate (200 mg per kg, subcutaneously) failed to inhibit the vessel wall cyclooxygenase. This suggests that cyclooxygenase activity at the site of inflammation may be uniquely sensitive to inhibition by sodium salicylate and perhaps other drugs. An alternative, although unlikely explanation, would be that sodium salicylate could selectively inhibit PGE₂ but not prostacyclin formation, by an action beyond the cyclooxygenase, perhaps by affecting the subsequent transformation of the endoperoxide intermediate.

The dual cyclooxygenase and lipoyxygenase inhibitor, BW755C, which also failed to inhibit mucosal cyclooxygenase activity, similarly caused little gastric damage. This again gives support to a relationship between the production of gastric erosions and the inhibition of mucosal prostacyclin formation by anti-inflammatory drugs. The doses of BW755C used in this study were 5–10 times greater than the anti-inflammatory doses required to reduce carrageenin foot paw oedema and white cell migration into sponges¹², suggesting a good therapeutic ratio for this experimental compound. Further, the dual inhibition of the two major pathways of arachidonate metabolism is likely to be a beneficial property in the control of leukocyte migration and inflammation¹².

Although cyclooxygenase enzyme preparations from tissues such as spleen, kidney and brain have shown differential sensitivities to some compounds *in vitro*¹⁶, the present findings clearly demonstrate that some non-steroid anti-inflammatory drugs can selectively inhibit prostaglandin biosynthesis in different tissues *in vivo*. The development of anti-inflammatory compounds which fail to inhibit cyclooxygenase in the gastric mucosa and lack topical irritancy seems to be a rational approach to obtaining clinically well-tolerated drugs.

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Lentil lectin effectively induces allotransplantation tolerance in mice

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The interaction of lectins with carbohydrate receptors on the plasma membrane of eukaryotic cells results in a wide variety of biological effects¹. One effect which has been extensively studied is the stimulation of lymphocytes to blastogenesis and proliferation. High doses of concanavalin A (Con A) and phytohaemagglutinin (PHA) have been shown to activate *in vitro* regulatory cells capable of suppressing proliferation of other cells in the culture^{2,3}. However, Con A and PHA treatment were used effectively to prolong the survival of skin and heart allografts^{4–8} before it was recognised that some lectins have an activatory effect on suppressor cells *in vitro*. A possible explanation of these tolerogenic effects is the activation of specific suppressor cells. In this letter we have compared systematically various lectins differing in their carbohydrate specificity and mitogenicity in relation to their ability to induce prolonged skin allograft survival in mice with the aim of selecting the most effective lectin treatment schedule. Some preliminary results of this study have been mentioned in a recent review⁹.

All lectins used were purified by affinity chromatography and were devoid of any electrophoretically detectable contaminants. Con A, pea lectin (PsA), lentil lectin (LcA) and sweet pea (*Lathyrus odoratus*) lectin (LoA) were prepared by affinity chromatography on Sephadex G-100 (refs 10–12), soybean lectin (SBA) by affinity chromatography on *N*-acetyl-D-galactosaminyl polyacrylamide gel¹³, *Erythrina indica* (EiA) lectin on α -D-galactosyl polyacrylamide gel¹⁴, wheat germ lectin (WGA) on *N*-acetyl- α -D-glucosaminyl polyacrylamide gel and scarlet runner (*Phaseolus coccineus*) seed lectin (PcA) by affinity chromatography on fetuin-Sepharose. Unless otherwise stated, mouse strain combination B10.D2 \times B10.D2(M504) (abbreviated to M504) was used; the histocompatibility difference between these strains lies in their H-2D regions^{15,16}. In other experiments another strain combination was used (B10.A \times C57BL10ScSn) (abbreviated to B10) these strains differing in all regions of the H-2 locus. To study the specificity of tolerance induced by the lectin treatment and B10.D2 skin graft in M504 recipients, third-party grafts from BALB/c mice were used. This strain is identical with the original donor strain (B10.D2) at the H-2 locus but differing at the multiple non-H-2 loci. In all cases, both recipients and donors were females.

Comparisons were made using three different time schedules of LcA injection relative to time of skin allografting (Fig. 1). In schedules where all 10 doses of the lectin were given before grafting or 5 doses were given before and 5 after grafting, allograft survival was not essentially affected. In contrast, 10 doses (each 250 μ g) given after grafting made 2 out of 6 animals long-term tolerant (skin graft survival >100 days). Further improvement of graft survival time could be achieved by increasing the lectin dose to 500 μ g and total number of doses given to 20. In these conditions allografts of all LcA-treated recipients survived for more than 100 days (Fig. 2). These long-term surviving grafts did not show any macroscopically visible signs of rejection.

The effect of different lectins was compared using this optimum schedule (20 doses of 500 μ g, all given intraperitoneally (i.p.) after transplantation) (Fig. 2). (The doses of Con A and WGA, however, were only 250 μ g because of their toxicity at higher doses.) WGA, SBA and EiA did not induce long-term

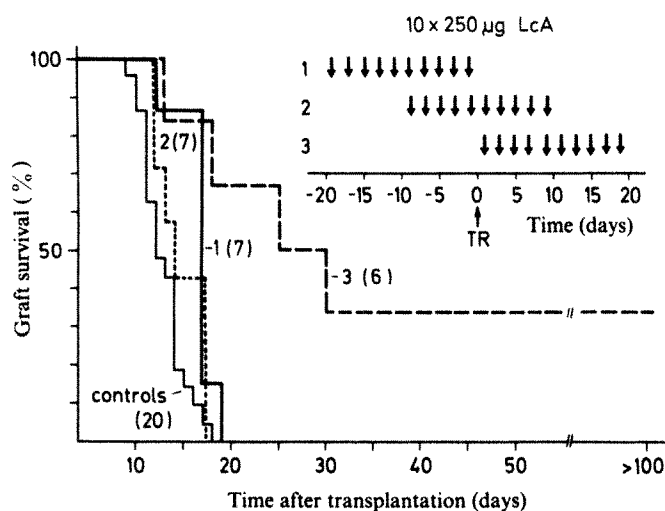


Fig. 1 Survival of B10.D2 grafts in M504 recipients treated with 10 doses of LcA (250 µg, i.p.). Comparison of three different time schedules of LcA administration. Inset shows the schedule of treatment (intervals between the doses were 48 h). Test transplantation (TR) is always on day 0. Skin grafts prepared from tail skin ~0.5 cm² in size were transplanted onto the backs of the tested recipients²⁵. Grafts with irreversible necrotic changes over their whole area were considered rejected.

skin allograft survival (date not shown), Con A, PcA and LoA induced tolerance only occasionally, whereas PsA induced long-term tolerance in about 50% of recipients and LcA in 100% of recipients. The LcA treatment did not seem to have any observable harmful side effects on the animals.

The efficiency of the most effective lectin, LcA, could be still increased by administering some doses intravenously (i.v.) (1st, 3rd, 5th and 7th dose given i.v., all others i.p.); in this situation, 20 doses of only 250 µg each induced long-term allograft survival in 90–100% of recipients. Long-term tolerance in 90–100% of recipients was induced also if the most effective lectin treatment started on the third day after transplantation; when it started 5 days after skin grafting, long-term tolerance was still induced in 50% of the cases.

Transplantation of a second B10.D2 allograft (no treatment) into recipients surviving with the first graft after 100 days as a result of LcA treatment led almost always to rejection of the

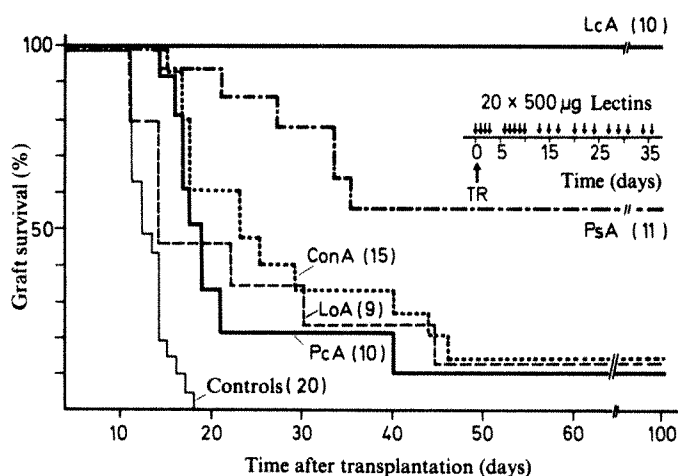


Fig. 2 Survival of B10.D2 grafts in M504 recipients treated with 20 x 500 µg i.p. doses of different lectins. The dose of Con A was 250 µg due its toxicity in higher doses. Inset time schedule of lectin treatment.

second graft in 12–20 days; the primary graft remained unaffected. This indicates either a rather unstable nature of the lectin-induced tolerance even 100 days after primary graft transplantation, or the occurrence of some form of protection of the first graft in the host. Although unexpected, this result is not unprecedented; similar effects were observed in the case of neonatally-induced tolerance (G. A. Voisin, personal communication) and in the case of liver extract-induced tolerance¹⁷, where the stability of tolerance was shown to increase progressively with time elapsed after transplantation (from 50th to 150th day). Studies on the possible stabilisation of lectin-induced tolerance with time and by increased lectin dose are now in progress in our laboratory.

Third-party test allografts (BALB/c) transplanted into recipients bearing a B10.D2 graft for >100 days resulted in a rapid rejection of the BALB/c graft (11–14 days), usually accompanied by a delayed rejection of the primary B10.D2 graft (in 15–30 days).

Therefore, the mechanism of this type of tolerance is not yet clear, but the lectin certainly does not act through nonspecific damage or suppression of immunological reactivity of the recipient as evidenced by the rapid rejection of third-party grafts. It is of interest that the lectin treatment is remarkably efficient even when it is started several days after transplantation; this indicates that the lectin might be interacting with the antigen-sensitised lymphocytes.

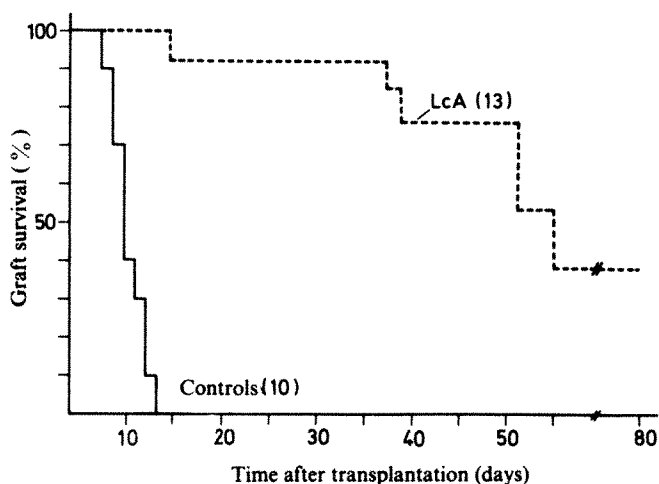


Fig. 3 Survival of B10.A grafts in B10 recipients treated with LcA. The lectin (1 mg in 0.5 ml phosphate buffered saline) was given i.p. daily starting with the day of transplantation (day 0) until rejection; the doses on days 1, 3, 5, 7 and 9 were given i.v. For other experimental details see Fig. 1 legend.

All lectins with detectable tolerogenic activity (except PcA) have similar carbohydrate specificity (α -D-Man, α -D-Glc) and are structurally fairly similar (a Con A-like group of lectins)¹⁸. Lectins with similar carbohydrate specificity and probably also similar structure are present in seeds of many other plants belonging to the Fabaceae family¹⁹; it is quite possible that some of these poorly studied lectins might be even more effective tolerance inducers than LcA.

The primary target of action of LcA and similar lectins is probably a specific carbohydrate receptor or class of receptors on the cell surface. However, the plant lectins might also act by mimicking the action of (or competing with) some endogenous lectin-like regulatory substances (for example, lymphokines).

The LcA treatment of recipients compares well with other treatments used for induction of adult transplantation tolerance (spleen extract alone²⁰ or in combination with hydrocortisone acetate²¹, antilymphocyte serum²²) at least in the mouse strain

combination tested (B10.D2×M504); unlike LcA, none of these treatments was able to induce long-term (permanent) survival of skin grafts in 100% of the recipients. Other highly effective ways of induction of transplantation tolerance in mice, for example combined treatment with several immunosuppressive drugs²³ or cyclosporin A treatment²⁴, were demonstrated in other strain combinations and thus direct comparison with LcA effects is not possible at present. Our recent results indicate that LcA is highly efficient in prolonging allograft survival and in inducing tolerance in a strain combination with a strong H-2 barrier (B10.A×B10) (Fig. 3).

In conclusion, LcA is possibly one of the most effective substances known so far (in combination with skin graft as a source of specific antigens) for inducing adult transplantation tolerance in mice. Experiments are now in progress to determine what maximal histocompatibility barrier can be overcome by LcA treatment alone or in combination with other immunosuppressive drugs, whether LcA is also effective in other animal species, the details of induction mechanism and maintenance of this type of tolerance.

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The human 'T' genetic region of the HLA linkage group is a polymorphism detected on lectin-activated lymphocytes

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The major histocompatibility complex of the mouse, H-2, comprises 12 loci which are grouped into regions and subregions¹. Present data indicate that to the 'right' of the H-2D there are nine loci clustered into one region, the 'T region'. These loci are designated Qa-1, Qa-2, Qa-3, Qa-4, Qa-5, H-2T, H-31, H-32, TLa (ref. 2). The loci in the 'T region' may, in fact, be part of the H-2 complex. This notion is supported by the fact that gene products of the TLa, Qa-2 and Qa-3 loci share some molecular properties with H-2 products: a two-polypeptide chain complex with a common determinant of molecular weight 12,000 β_2 -microglobulin and a variable heavy chain of MW 45,000 (ref. 3). Recent reports suggested the possible existence of human analogues to the gene products of the murine 'T region'. Antigenic structures, similar but not identical with HLA-A, -B and -C antigens, were recently isolated from the human lymphoblastoid T-cell line MOLT4 (refs 4, 5). These reports presented evidence for the existence of a β_2 -microglobulin or a β_2 -microglobulin-like molecule, Bt, associated with a polypeptide chain of MW ~ 45,000. However, it remains to be determined whether these structures are equivalent to the mouse Qa-1, Qa-2 or TLa gene products. We now report the identification of human alloantisera that react with cell-surface determinants expressed on mitogen-activated T lymphocytes but not expressed on resting T cells. Antigen blocking and modulation techniques were used. The alloantigens identified (HT) are different from the classical HLA-A, -B, -C and -DR antigens but are associated with β_2 -microglobulin. These determinants are encoded by genes in the HLA-A region and may represent the human counterpart of the TLa region determinants of the mouse^{1–3}.

We have screened 200 sera obtained from women with a history of multiple pregnancies. The sera were exhaustively absorbed with platelets and were shown to be non-reactive with HLA-A, -B and -C antigens carried by the resting peripheral blood lymphocytes from two different individuals: D.W., whose HLA phenotype is A1, A3, B8, B18, Cw2, DRw3, DRw5; and E.G., whose HLA phenotype is A2, A3, B14, B37, Cw6, DRw1 and DRw5. Lectin (phytohaemagglutinin (PHA) or concanavalin A (Con A))-activated lymphocytes obtained from these two individuals were used to screen the platelet-absorbed pregnancy sera. Two antisera, SF48 and K, were found to react exclusively with the activated lymphocytes by a cytotoxicity score of 40–60%.

The sera were subsequently absorbed with resting lymphocytes from either D.W. or E.G. by a tray microabsorption technique (200×10^6 lymphocytes per ml) and the sera were found to retain their reactivity with mitogen-activated lymphocytes only (Table 1). The sera were then tested in a random panel of activated lymphocytes and gave 11/23 (47.8%) and 8/23 (34.8%) positive reactions with SF48 and K, respectively. The 2×2 tables between the HLA assignment of the panel and the cytotoxic reactions of these two sera are given in Table 2. Significant associations were found for reactivities of serum SF48 with HLA-A3 ($P = 1.77 \times 10^{-6}$) and serum K with HLA-A1 ($P = 1.24 \times 10^{-3}$). Having serologically ruled out the existence of antibodies against HLA antigens, we tested these sera in a selected panel of HLA-A1 or HLA-A3 phenotypes and demonstrated that lectin-activated lymphocytes in three out of five HLA-A1 individuals reacted with K, and five out of seven HLA-A3 individuals reacted with SF48 (Table 3). In addition, three T-lymphoblastoid cell lines were tested with SF48 and K antisera: HSB-2 (HLA-A1, A2, B17, Bw44), RPMI-8402 (HLA-A1, A29, B18, B16) and MOLT-4 (HLA-A1, A10). Only MOLT-4 was able to absorb both the SF48 and K reactivity directed against D.W. and E.G. test cells, but did not remove the reactivity of HLA-A3 antiserum. MOLT-4, in contrast to the activated lymphocytes (see Table 1), reacted with 100% cytotoxicity, indicating that all MOLT-4 cells carry the antigen(s) detected by SF48 and K. It is of interest that the MOLT-4 cells have recently been shown to contain antigenic structures different from HLA-A and -B but associated with β_2 -microglobulin^{4,5}. These experiments, *per se*, exclude the possibility that the K and SF48 antigenic determinants represent a presentation of HLA-A, -B or -C antigens that are unique to lectin-stimulated lymphocytes.

To examine the remote possibility that HLA antibodies were not removed from SF48 and K sera after sequential absorptions

Table 1 Serological characterisation of PHA- and Con A-activated lymphocytes

Donor	Cell tested	Pretreatment	Microcytotoxicity scores with			
			Alloantisera		Heteroantisera	
			SF48	K	goat anti- β_2 m	rabbit anti p29,34(C91)
D.W.	E ⁺ lymphocytes	—	1	1	8	1
	E ⁻ lymphocytes	—	1	1	8	8
	PHA-activated lymphocytes	—	6	6	8	4
	Con A-activated lymphocytes	—	6	6	8	4
	Absorption of sera on:					
	PHA-activated lymphocytes	lymphocytes	6	6	1	1
	PHA-activated lymphocytes	E ⁺ lymphocytes	6	6	1	4
	PHA-activated lymphocytes	E ⁻ lymphocytes	6	6	1	1
	Blocking with:					
	PHA-activated lymphocytes	Turkey anti-p29,34	6	6	8	1
	PHA-activated lymphocytes	Turkey anti- β_2 m	2	1	1	4
	Con A-activated lymphocytes	Turkey anti-p29,34	6	6	8	1
	Con A-activated lymphocytes	Turkey anti- β_2 m	2	1	1	4
E.G.	E ⁺ lymphocytes	—	1	1	8	1
	E ⁻ lymphocytes	—	1	1	8	8
	PHA-activated lymphocytes	—	4	1	8	4
	Con A-activated lymphocytes	—	4	1	8	4
	Absorption of sera on:					
	PHA-activated lymphocytes	Lymphocytes	4	1	1	1
	PHA-activated lymphocytes	E ⁺ lymphocytes	4	1	1	4
	PHA-activated lymphocytes	E ⁻ lymphocytes	4	1	1	1
	Blocking with:					
	PHA-activated lymphocytes	Turkey anti-p29,34	4	1	8	1
	PHA-activated lymphocytes	Turkey anti- β_2 m	1	1	1	4
	Con A-activated lymphocytes	Turkey anti-p29,34	4	1	8	1
	Con A-activated lymphocytes	Turkey anti- β_2 m	1	1	1	4

The platelet-absorbed pregnancy sera SF48 and K were tested in a microcytotoxicity assay, using fluorescein diacetate¹². The numbers are estimates obtained in the two-stage microlymphocytotoxicity assay: 1, 2, 4, 6 and 8 denote 0%, 10–25%, 25–50%, 50–75% and 75–100% cytotoxicity above background, respectively. Peripheral blood lymphocytes were separated on a Ficoll-Hypaque density gradient, washed three times and cultured at 37 °C in RPMI-1640 containing 20% pooled human serum, penicillin, streptomycin and L-glutamine. The cells were cultured for 5 d at a cell density of 1×10^6 per ml with either 5 μ g Con A per ml or 1.201 PHA per ml. E⁺ and E⁻ lymphocytes were obtained after rosetting with neuraminidase-treated sheep red blood cells¹³. Absorptions were carried out with 50,000 cells per μ l of serum in the case of E⁺ and E⁻ cells and with 200,000 cells per μ l of serum when whole lymphocytes were used. Blocking was done as previously published¹⁴. Turkey and goat-anti- β_2 -microglobulin (β_2 m) sera were prepared by multiple injections (in complete Freund's adjuvant) of β_2 -microglobulin purified from the urine of kidney transplant patients¹⁵. Turkey and rabbit (C91) anti-p29,34 (anti-Ia) sera were prepared in a similar way. The p29,34 antigens were isolated from a highly purified plasma membrane preparation¹⁶ of the B-lymphoblastoid cell line JY after solubilisation in deoxycholate by lentil lectin affinity chromatography and anti- β_2 -microglobulin immunoabsorbent column chromatography as described earlier^{17,18}. All sera were heat inactivated before use.

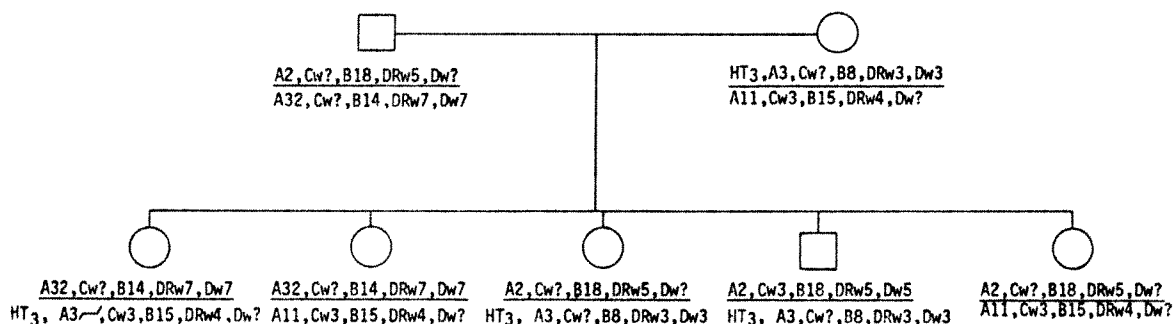


Fig. 1 A family (family F) using a microcytotoxicity assay with serum SF48. The first sibling on the left is an HLA-A/B recombinant. This family was recently discovered and has been thoroughly studied in the 8th International Histocompatibility Workshop.

with pooled platelets and resting lymphocytes from the same donor of the activated lymphocytes, the following experiment was carried out. Activated lymphocytes from individuals who were: (1) HLA-A3⁺, SF48⁺, (2) HLA-A3⁺, SF48⁻, and (3) HLA-A3⁻, SF48⁻, were used in cross-absorption experiments, in which HLA alloantisera defining HLA-A3 and SF48 were tested. The results obtained indicated that HLA-A3⁺, SF48⁺

cells absorbed all activity from both types of antisera, HLA-A3⁺, SF48⁻ cells absorbed the HLA only and HLA-A3⁻, SF48⁻ cells absorbed neither of them (Table 4).

Second, antigenic modulation experiments were carried out using a rabbit anti-HLA heavy chain serum, C41. For the preparation of the rabbit anti-HLA heavy chain antiserum (C41), the larger polypeptide chain of papain-solubilised HLA-

Table 2 2×2 tables of association between HLA assignment of the panel and SF48 and K reactivity

	HLA-A3		K	HLA-A1	
	+	-		+	-
	+11	1		4	4
SF48	- 0	11		- 0	15
$P(\text{Fisher}) = 1.77 \times 10^{-6}$				$P(\text{Fisher}) = 1.24 \times 10^{-3}$	

HLA assignment of the screening panel compared with the reactivity to SF48 and K. The panel consisted of unrelated randomly chosen individuals ($n = 23$). The P value reflects the probability of a random association between the two variables under study.

A2 antigen was isolated from the B-lymphoblastoid cell line RPMI-4265 as described elsewhere⁶. The resulting antibody had a cytotoxicity titre of 1:100 using lymphoblastoid cell lines JY and 1:10 using resting peripheral lymphocytes. This antibody also reacted with Daudi cells which agrees with studies showing that Daudi cells express the heavy chain of HLA antigens on their surface⁷. In addition, two monoclonal HLA heavy chain antibodies (w6/32 and w34/28)⁸ were obtained commercially (from Accurate Chemical Corporation). Both resting and mitogen-activated lymphocytes were incubated with varying dilutions of these antisera for periods extending to 6 h. The lymphocytes were then washed and retested with the three anti-HLA heavy chain sera or with alloantibodies. Results showed that prior incubation of the activated lymphocyte with HLA heavy chain antibody, in the absence of complement, did not suppress HLA heavy chain determinants on retesting in a subsequent cytotoxicity assay. Indeed, all three HLA heavy chain antibodies, in addition to HLA-A1, HLA-A3, SF48 and K antisera, were still capable of lysing the test cells. However, antigenic modulation of activated lymphocytes from E.G. (A2, A3, B14, B37, Cw6) with HLA-A3 antibody (kras) eliminated subsequent lysis mediated by HLA-A3 and complement, but not lysis by SF48 and complement. Following antigenic modulation with HLA-A3 antibody in control experiments, subsequent lysis was positive with HLA-A2 and HLA-B14 antisera but negative with HLA-A1 antiserum and complement. These results indicated that SF48 and HLA-A3 membrane antigens are expressed independently on the cell surface of mitogen-activated lymphocytes.

As Ia antigens are expressed on activated T cells⁹⁻¹¹, we designed experiments to determine whether or not the reactivity in SF48 and K alloantisera is due to antibody directed against DRw antigens. Blocking experiments were carried out by pretreating Con A- and PHA-activated T cells with a turkey

anti-Ia (p29, 34) serum and subsequently testing K and SF48 alloantisera in a microcytotoxicity assay¹² (Table 1). This turkey antiserum, which does not bind mammalian complement, completely eliminated the reactivity with eight specific anti-DRw allosera (data not shown) and a rabbit anti-p29,34 serum C91 (Table 1). However, the reactivity of K and SF48 alloantisera was not affected by prior blocking with the turkey anti-Ia serum. These results indicated that the reactivity of K and SF48 alloantisera is not directed against DRw Ia-like determinants on the activated lymphocytes.

Taken together, these experiments indicated that PHA- and Con A-activated lymphocytes contain antigenic determinants that are associated with β_2 -microglobulin but are different from the classical HLA-A, -B and -C antigens. Serological studies on a random panel showed that these determinants are genetically related to the -A1 and -A3 alleles of the HLA-A locus, but as shown in Table 3, some false negative and false positive assignments can be made, indicating a serological independence of the K and SF48 specificities from the HLA-A1 and -A3 determinants. Genetic analyses of four families with informative segregation for both SF48 and K in microcytotoxicity assays indicated linkage of these determinants with HLA. One of these families (Fig. 1) demonstrated linkage between the locus coding for HLA-A and that coding for the antigenic determinant(s) recognised by SF48 alloantiserum.

Table 4 Cross-absorption with PHA-activated lymphocytes

Antisera	Absorbed with:		
	A3 ⁺ SF48 ⁺	A3 ⁺ SF48 ⁻	A3 ⁻ SF48 ⁻
Anti-HLA-A3	1	1	8
SF48	1	6	6
Testing cell = A3 ⁺ SF48 ⁺			

All absorptions and cytotoxicity scoring are as in Table 1. HLA phenotype of activated lymphocytes from the A3⁺, SF48⁺ individual D.W. is A1, A3, B8, B18, Cw2, from the A3⁺, SF48⁻ individual J.S. is A3, A24, B7, B17, and from the A3⁻, SF48⁻ individual D.F. is A11, Aw32, B14.1, B15.1, Cw3, Bw6.

We conclude that alloantisera K and SF48 detected antigenic determinants of a new polymorphic locus closely linked to the HLA-A locus on chromosome 6 in man. We have tentatively named it HT (human T locus) and have described two possible alleles, HT-1 and HT-3, based on the high degree of association and/or linkage disequilibrium with HLA-A1 and HLA-A3.

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Table 3 Comparison of HLA and DR assignments to those of SF48 and K reactivities in a selected screening panel

Individuals	HLA	DRw	SF48	K
T.R.	A2, A3, B17, B18	7	+	+
K.F.	A1, A1, B8, B8	3,3	+	+
B.M.	A3, Aw32, B8, Bw35	3,4	+	+
B.F.	A11, Aw32, B13, B15	5,7	-	+
A.M.	A2, A3, B7, B18	1,2	+	+
J.S.	A3, A24, B7, B17	4,7	-	+
L.M.	A1, A2, B8, B15	NT	+	-
M.M.	A1, A3, B7, Bw52	NT	+	-
F.Y.	A3, A26, B5, B38	NT	+	+
B.G.	A1, A29, B14, B17	NT	+	+
D.C.	A2, A3, B7, B-	2,3	-	-
L.A.	A1, A2, B8, Bw40	1,3	-	-

PHA-activated lymphocytes from a selected panel of HLA-A1, HLA-A3 positive individuals. Those listed were selected from our regular HLA-screened panel and have been typed several times for study in two international workshops. NT, not tested.

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T-cell-derived helper factor allows *in vivo* induction of cytotoxic T cells in *nu/nu* mice

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T-cell immunocompetence and diversity are thought to be generated in the thymus^{1,2}. This view is based on the findings that (1) T-cell ontogeny is thymus dependent^{3,4}, (2) the major histocompatibility restrictions of T-cell interactions are phenotypically related to the H-2 type of the thymus⁵⁻⁹, and (3) the phenotypic manifestation of H-2-linked immune responsiveness parallels the restriction elements selected in thymus¹⁰⁻¹². However, it is unclear whether pre-thymic cells programmed to develop into T cells do already express a receptor diversity, also whether pre-thymic cells have the potential to react against self-antigens, and whether the mechanism of self-tolerance is initiated in the thymus by either elimination or suppression of self-reactive clones. If it were possible to confer on pre-thymic cells antigen-specific effector functions, the impact of the thymus on the generation of T-cell diversity and function could be analysed in more detail. In mice, the nude mutation lacks a functioning thymus^{13,14}; *nu/nu* mice possess a thymic rudiment which is epithelial in the embryo¹⁵ and a fibrous, cystic remnant in adult^{15,16}; this remnant is not populated by lymphoid cells¹⁵⁻¹⁷. At present, the absence of immunocompetent T cells in *nu/nu* mice is explained by a lack of thymic differentiation and maturation of pre-thymic cells (reviewed in ref. 13). Here we report that injection of allogeneic stimulator cells plus a Lyt 1 T-cell-derived helper factor^{18,19}, termed interleukin 2 (for the system of nomenclature, see ref. 20) allows lymphocytes of *nu/nu* mice to differentiate *in vivo* into alloreactive cytotoxic T lymphocytes (CTLs).

The rationale of our experiments is based on the following observations. Murine thymocytes generate *in vitro* little, if any, cytolytic activity in response to otherwise immunogenic stimuli. However, in the presence of interleukin 2, the cytolytic response generated to alloantigens is almost equal to that normally existing in peripheral T cells²¹. More recently, we noted that even peanut-agglutinin-positive thymocytes, assumed to be cortical cells and thus immunoincompetent²², contained high numbers of CTL precursors which develop into CTLs in the presence of interleukin 2 (ref. 23). This suggested that the limiting factor in antigen-induced cytotoxic responses of immature thymocytes is not a lack of antigen-reactive CTL precursor cells, but a relative lack of cells producing interleukin

2. As cortical thymocytes had been assumed to be immunoincompetent, but exhibited immunocompetence in the presence of interleukin 2 (ref. 23), it was of interest to test whether in the presence of alloantigen interleukin 2 could also confer T-cell competence to lymphocytes in thymus-deficient *nu/nu* mice. The second observation relevant in this context was that sera of thymus-bearing normal mice inhibited the *in vitro* activity of the Lyt 1 T-cell-derived interleukin 2. In contrast, sera from athymic mice supported its functional activity (C.H., unpublished data). It was thus reasonable to assume that on injection of interleukin 2 into athymic mice, its functional activity would be maintained. Consequently, we tested whether *in vivo* T-cell precursors of *nu/nu* mice would respond to alloantigens, provided semi-purified¹⁸ interleukin 2 was simultaneously injected.

Of the various experimental approaches tested so far, reproducible results were obtained using the following protocols. Protocol A: On two consecutive days, 2×10^7 irradiated (2,000 rad) C57BL/6 mouse-derived splenic stimulator cells (H-2^b) were injected into the hind footpad of BALB/c *nu/nu* mice. Starting at the same time, the mice received, subcutaneously into the hind footpad, 100 μ l of helper factor twice a day for 3 d. Protocol B: BALB/c *nu/nu* mice received at the same time intervals the same dose of stimulator cells and helper factor intravenously. At day 5, the mice were killed and their lymph node cells and splenic cells tested for cytotoxic activity towards H-2^b, H-2^k and H-2^d target cells. As can be seen from Table 1, both protocols resulted in the *in vivo* induction of antigen-specific cytolytic effector cells within the spleen cells of *nu/nu* mice.

We subsequently defined a more convenient protocol for generating alloreactive cytolytic effector T cells from *nu/nu*-derived precursor T cells. Because the Lyt 1 T-cell-derived factor is generated in the course of a mixed lymphocyte culture, we reasoned that the factor ought also to be produced in the course of a graft-versus-host reaction (GvH) *in vivo*. Moreover, Piguet and Vasalli²⁴ recently reported that *nu/nu* mice are apparently able to reject up to 6×10^7 grafted allogeneic cells, provided the grafted cells contained T lymphocytes. This apparent paradoxical finding was not pursued further by the authors. One explanation for this is that during a T-cell-dependent GvH reaction induced in nude mice, helper factor may be produced *in vivo*, which in turn would allow *nu/nu* T-cell precursors to respond to the immunogenic stimulus represented by the grafted allogeneic cells.

In agreement with the results of Piguet and Vasalli²⁴, we noted that 8–9 d after injection of 6×10^7 allogeneic spleen cells the recipient *nu/nu* mice had rejected the grafted cells, that is, no grafted cells were detectable by serological techniques in the spleen of the killed recipient *nu/nu* mice (data not given). More interestingly, 8 d after grafting, the recipient *nu/nu* spleen cells exhibited low but significant cytolytic activity with specificity for target cells expressing the same alloantigen as the grafted cells (Table 2). The magnitude of cytolytic activity could be enhanced by restimulation of the *nu/nu* spleen cells *in vitro* in the presence

Table 1 *In vivo* induction of antigen-specific cytolytic effector cells in thymus-deficient nude mice

Protocol used	Cells tested	EL4 (H-2 ^b)			% Specific lysis of target cells			P815 (H-2 ^d)		
		500:1	120:1	30:1*	500:1	120:1	30:1	500:1	120:1	30:1
A	LN	12	9	2	1	0	1	–3	1	0
	spleen	31	10	3	0	–1	–1	8	6	3
B	LN	7	3	3	1	0	–1	–3	1	N.D.
	spleen	22	10	2	–1	3	2	2	–2	1

Protocol A: BALB/c nude mice (Bomholtgaard) were injected on two consecutive days, each with 2×10^7 C57BL/6-derived, irradiated (2,000 rad) splenic lymphocytes into the hind footpad. Simultaneously, the mice were injected at the same site, twice a day for 3 d, with 100 μ l semi-purified T-helper factor¹⁸. Protocol B: at identical time intervals, BALB/c nude mice received intravenously the same dose of stimulator cells and T-helper factor as in A. After 5 d, the mice were killed and single cell suspensions were prepared from either lymph node cells (LN) or spleen cells¹⁸. Lymphocytes were assayed for cytolytic activity in a 4-h standard ⁵¹Cr-release assay towards EL 4(H-2^b), LS (H-2^k) and P815 (H-2^d) target cells essentially as described elsewhere¹⁸. For each population tested, a dose-response curve was established. Per cent specific ⁵¹Cr-release was calculated according to the formula described previously¹⁸. Background lysis of the target cells was less than 23%.

* Ratio of effector cells to target cells used in the 4-h ⁵¹Cr-release assay.

Table 2 GvH reaction-induced sensitisation of alloreactive CTL in nude mice

Effector cells from	<i>In vivo</i> restimulation (5 d)	Treatment of effector cells	P815 (H-2 ^d)			% Specific lysis of targets EL4 (H-2 ^b)			LS (H-2 ^k)		
			300:1	60:1	12:1*	300:1	60:1	12:1	300:1	60:1	12:1
BALB/c (nudes) grafted with C57BL/6 spleen cells (H-2 ^b) for 8 d	ND	ND	4	2	—	15	13	6	1	—1	—
	H-2 ^b stimulator cells plus T-helper factor	NMS + C'	9	2	0	59	48	23	—1	0	—
		anti-Thy 1 + C'		ND		3	0	0		ND	
BALB/c (nudes) grafted with CBA spleen cells (H-2 ^k) for 8 d	ND	ND	4	0	2	3	2	—1	16	9	3
	H-2 ^k stimulator cells plus T-helper factor	NMS + C'	0	0	0	4	3	0	25	19	6
		anti-Thy 1 + C'		ND			ND		1	0	1

Thymus-deficient BALB/c mice received intravenously either 6×10^7 C57BL/6 mouse (H-2^b) or CBA mouse (H-2^k) splenic lymphocytes. After 8 d, the mice were killed and single cell suspensions were prepared from the spleen cells. According to the results obtained in complement-dependent cytotoxicity assay using anti-H-2^k, anti-H-2^d, anti-H-2^b antisera⁸, more than 95% of the spleen cells were BALB/c-mouse derived. One part of the spleen cells was assayed for cytotoxicity against ⁵¹Cr-labelled P815 (H-2^d), EL4 (H-2^b) and LS (H-2^k) target cells in a 4-h ⁵¹Cr assay. The remaining spleen cells (4×10^6) were cultured *in vitro* for an additional 5 d in the presence of irradiated stimulator cells (1×10^6) plus 50 μ l semi-purified T-helper factors¹⁸ in conditions described previously^{18,23}. Thereafter, the cells were treated with normal AKR serum plus complement or with monoclonal anti-Thy 1.2 antibody (derived from clone F7D5; Olac) plus C. The surviving cells were assayed for cytotoxicity against the target cells listed. Background lysis of the target cells during the 4 h ⁵¹Cr-assay did not exceed 20%.

* Ratio of effector to target cells. ND, not done.

of T-helper factor (Table 2). As the cytolytic activity was abrogated after treatment of the cells with anti-Thy 1 antisera plus complement (Table 2), we concluded that the antigen-specific cytolytic activity generated *in vivo* was carried out by *nu/nu* mouse-derived T cells.

Despite the presence of some Thy 1-positive cells²⁵ and possibly ectopic thymus tissue²⁶, normal *nu/nu* mice exhibit no T-cell functions^{27,28}. Although an increased *in vitro* development of Thy 1 antigen on the surface of nude spleen cells has been described following treatment with thymus extracts^{29,30}, culturing on thymus reticuloendothelial cell monolayers³¹ or exposing the cells to neuraminidase³² or poly(AU) (ref. 33), appearance of the Thy 1 surface antigen should only be equated with T-cell differentiation if T-cell function is demonstrated. This has been achieved recently by Gillis *et al.*³⁴. Accordingly, lymphocytes from *nu/nu* mice were able to differentiate *in vitro* into alloreactive CTLs, provided the cells were cultured in the presence of stimulator cells plus T-cell growth factor (TCGF). Obviously, these *in vitro* results almost parallel our *in vivo* findings described here.

Our results are relevant to two issues. First, a well-characterised lymphokine^{18,19} is now shown to be active *in vivo* in restoring T-cell immune functions in *nu/nu* mice. Second, the data imply that *nu/nu* mice contain lymphoid cells which can be activated *in vivo* by an allogeneic stimulus and interleukin 2 to generate cytotoxic T cells.

Recent work has shown that interleukin 2 is released from Lyt 1 T cells in response to allogeneic and mitogenic stimuli¹⁸. *In vitro*, interleukin 2 replaces the Lyt 1 T-helper cell requirement of thymic Lyt 123 CTL precursors, thus allowing the latter to differentiate in response to antigenic stimuli into alloreactive of H-2-restricted Lyt 23 CTLs (ref. 23 and C.H. *et al.*, in preparation). Systemic *in vivo* application of interleukin 2 plus alloantigen is shown here to trigger in otherwise T-cell immunoincompetent *nu/nu* mice the induction of alloreactive CTLs. Therefore, *nu/nu* mice must possess interleukin 2-sensitive T-precursor cells endowed with the receptor repertoire required for alloreactivity.

One could argue that the thymic rudiment of *nu/nu* mice induces T-cell lymphopoiesis with a functional activity below normal detection. This low activity might be boosted by the *in vivo* activity of interleukin 2 to the levels described here. If so, *in vivo* application of interleukin 2 might be useful as an immunopharmacological agent for amplifying weak T-cell reactivity *in vivo*. If, however, the thymus defect in *nu/nu* mice is absolute, the results imply that interleukin 2 bypasses the requirement of a thymus during the *in vivo* differentiation of pre-thymocytes into

alloreactive CTLs. According to this reasoning, pre-thymic cells already contain the receptor repertoire required for alloreactivity. We do not know whether the repertoire for alloreactivity is dependent on pre-thymic somatic events or whether it is inherited from the germ line, as suggested by Jerne². Because the repertoire for alloreactivity might already be expressed on pre-thymic cells, and because alloreactive and H-2-restricted CTLs may be identical^{35,36}, it is also possible that the repertoire for extrinsic foreign antigens is expressed in pre-thymic precursor T cells. If so, we envisage, at the level of precursor T cells, distinct clones with the potential to react to an extrinsic antigen in the context of both syngeneic and allogeneic H-2 structures.

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Note added in proof: Using the very same protocol we recently succeeded in inducing in *nu/nu* mice T helper cells specific for heterologous sheep erythrocytes (Stötter, Rude and Wayne, submitted for publication).

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Chemical characterisation of the Fab and Fc fragments from surface immunoglobulin

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Immunoglobulin (Ig) molecules of the M and D classes are present on the membranes of B lymphocytes (sIg), where they serve as antigen receptors^{1–6}. sIg is a biosynthetically stable membrane protein which requires either denaturing conditions or detergents to free it from other membrane constituents^{1,7–9}. Whether the right association of sIg with the membrane is due to the chemical nature of the sIg molecule itself or to another constituent of the membrane, and the mechanism by which the recognition of antigen becomes a signal for cell division and differentiation, have become topics of current interest. Several mechanisms by which the membrane-sIg association could be maintained have been proposed^{10–13}: the sIg could have a unique peptide structure and/or amino acid sequence responsible for stabilising the association; the carbohydrate composition of sIg could impart physical and/or chemical characteristics which maintain the association; sIg could be closely associated with another protein, the proreceptor, which would be responsible for holding the sIg in the membrane. Here, we use charge-shift electrophoresis¹⁴ to examine the detergent-binding properties of mouse sIgM and sIgD and the tryptic Fab and Fc fragments derived from these molecules. In this technique, hydrophobic proteins which bind detergent are recognised by an altered electrophoretic behaviour in the presence of charged detergents. Proteins which fail to bind detergent do not show a 'shift' in electrophoretic migration. Our results confirm the observation that sIg is a hydrophobic protein which binds detergent^{15–17}, and demonstrate that the site of detergent binding is located in the C-terminal portion of the sIg heavy chain.

Suspensions of murine splenocytes were radioiodinated and the labelled surface proteins solubilised by detergent lysis^{18,19}. sIgM, sIgD and the Fab and Fc fragments were isolated by a combination of affinity chromatography and tryptic digestion (Fig. 1). The ¹²⁵I-labelled lysate was divided into two aliquots. One aliquot was passed over an anti- μ affinity column; complete sIgM molecules were subsequently eluted from this column. The unbound material contained ¹²⁵I-sIgD in addition to other labelled surface proteins. This eluate was digested with trypsin in conditions previously shown to give complete cleavage of sIgD into Fab and Fc fragments²⁰. The digest was applied to an anti- δ affinity column from which sIgD Fc fragments were eluted. The digest, depleted of Fc fragments, was passed over an anti-light-chain column from which the sIgD Fab fragments were subsequently obtained.

The second aliquot was subjected to a similar procedure except that the anti- δ affinity column preceded, and the anti- μ affinity column followed, the proteolytic digestion. The hydrophobicity of the sIg molecules and tryptic fragments was examined by charge-shift electrophoresis¹⁴ (Fig. 2) and gave the following results. Neither the myeloma proteins, run as standards, nor the sIg or sIg fragments moved from the origin in the presence of the non-ionic detergent Triton X-100. In the presence of the anionic detergent deoxycholate, anodal migra-

tion of the sIgM, sIgD, sIgM Fc and sIgD Fc fragments was observed.

These results demonstrated that the Fc fragments of sIg contain the hydrophobic regions responsible for detergent binding. Perhaps the simplest explanation for these observations is that sIgM and sIgD have a unique stretch of hydrophobic amino acids at, or near to, the C-terminus of the heavy chain. The recent experiments of Williams *et al.*²¹ support this hypothesis, although the earlier experiments of Walsh and Crumpton²² and McIlhinney *et al.*²³ lead to a contrasting, although unproven conclusion.

Another possibility is that the hydrophobicity was due to incomplete glycosylation of the sIg. Several arguments against a relationship between the extent of glycosylation and detergent binding can be made. First, removal of carbohydrate residues from secreted human IgM myeloma proteins with neuraminidase and β -galactosidase did not result in an appearance of detergent-binding properties (J.L., unpublished observation). Second, secreted IgG does not contain a sub-fraction which binds detergent¹⁴, although the carbohydrate composition of these molecules is known to be heterogeneous²⁴. Third, other integral membrane proteins, such as the murine and human histocompatibility antigens, are highly glycosylated, hydrophobic and capable of binding detergent. Finally, intracellular 7S IgM from mouse myeloma cells, although deficient in carbohydrate²⁵, does not bind detergent in the charge-shift electrophoresis assay (R.M.E.P., unpublished work).

It is possible that the hydrophobicity of sIg was actually due to a closely associated, noncovalently bound hydrophobic proreceptor undetected by the iodination procedures. This possibility is less likely in view of the observations that biosynthetically radiolabelled intracellular Ig from the murine B-cell leukaemia 70Z/3 (J.L. and C. J. Paige, manuscript in preparation) or from chicken bursal cells²⁶ showed the same electrophoretic behaviour as we report for murine sIgM and sIgD. This biosynthetically labelled Ig was free of peptides other than heavy and light chains when the cells were solubilised with both NP-40 and 2% sodium deoxycholate^{13,27}.

Although not conclusive, our results are consistent with the interpretation that the hydrophobic nature of sIg is sufficient to account for the stability of the plasma membrane-sIg asso-

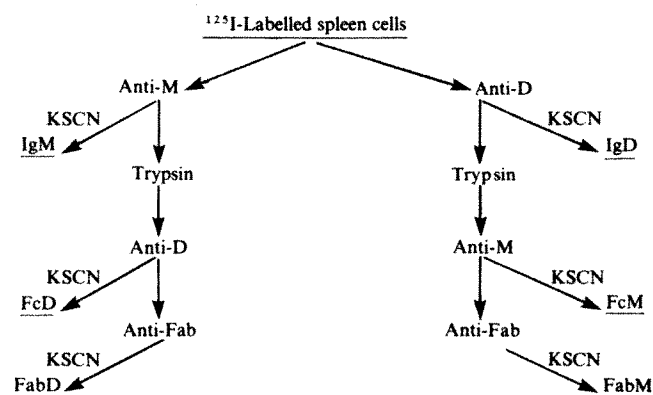


Fig. 1 Preparation of complete and tryptic cleaved sIg. The lysate was divided into two aliquots and subjected to the first stage of affinity chromatography as described in the text. The sIgD-containing lysate was digested with TPCK-trypsin (Sigma) at 0.1 mg ml⁻¹ for 30 min at room temperature. The sIgM-containing lysate was treated identically except that incubation was at 37 °C for 3 h. The digestion was stopped by the addition of 1 mg ml⁻¹ soybean trypsin inhibitor, 1 mg ml⁻¹ egg white trypsin inhibitor and 1 mM phenylmethylsulphonyl fluoride. The second and third stages of affinity chromatography followed. Material bound to the affinity resins was eluted with 4 M KSCN containing 0.5% Triton X-100 after washing the column with 0.5% Nonidet P-40 phosphate-buffered iodine, pH 7.4, and four column volumes of 1 M KSCN containing 0.5% Triton X-100. The samples were dialysed against 0.05 M glycine-NaOH, 0.1 M, NaCl and 0.5% Triton X-100, pH 9.0.

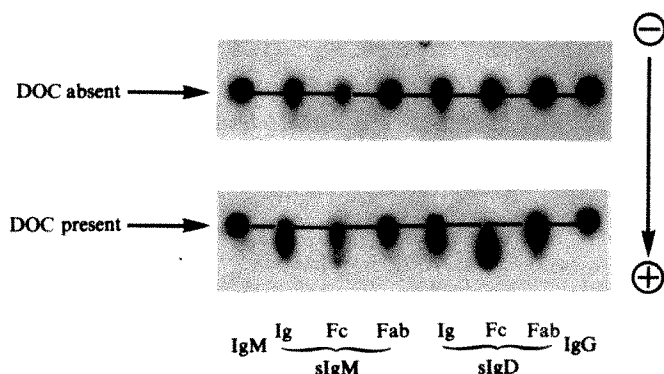


Fig. 2 Charge-shift electrophoresis of sIg and tryptic fragments. Rabbit antisera to mouse μ -chains, δ -chains and κ -chains²⁸ were precipitated at 1.6 M ammonium sulphate and the immunoglobulin fractions coupled to CH-Sepharose 4B (Pharmacia) using carbodiimide according to the manufacturer's instructions. The anti- μ and anti- δ sera were without specificity towards the Fab portions of the molecules. Spleen cells from male CBA mice were radioiodinated by the lactoperoxidase-catalysed procedure and lysed in 0.5% (w/v) Nonidet P-40^{18,19}. The nuclei and cellular debris were removed by centrifugation at 15,000g for 15 min, 0 °C, and the free ¹²⁵I removed by chromatography on Sephadex G-25. sIgM, sIgD and their respective Fc fragments were prepared as described in Fig. 1 legend. Charge-shift electrophoresis in 1% agarose (w/v) gels containing either 0.5% Triton X-100 or 0.5% Triton X-100 plus 0.25% (w/v) sodium deoxycholate was carried out as described by Helenius and Simons¹⁴. Sodium deoxycholate, 2%, was added to the samples at least 5 h before electrophoresis. Gels were fixed in methanol/acetic acid/water, 1:1:8, and dried onto chromatography paper. Autoradiography using pre-flashed film and fast tungstate intensifying screens was carried out according to the method of Laskey and Mills²⁹. In the bottom panel, the anodal migration of sIgM, sIgD and their respective Fc fragments is evident. The ¹²⁵I-labelled serum IgM and IgG samples were purified mouse myeloma proteins MOPC 104E and Adj PCS, respectively. The migration of these samples with or without sodium deoxycholate was unaffected by prior chemical reduction with dithiothreitol or treatment with 4 M KCNS and dialysis against the electrophoresis buffer.

ciation. The contribution of the Fc-localised hydrophobic region of sIg to the mechanism of signal transduction, whether the sIg is a transmembrane protein, and the structural differences between sIg and serum immunoglobulin of the same class, remain to be determined.

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Inherited defect in a Na⁺, K⁺-co-transport system in erythrocytes from essential hypertensive patients

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The Na⁺ and K⁺ electrochemical gradients across cell membranes are believed to be maintained by the action of a Na⁺, K⁺-pump¹⁻³. In human erythrocytes this pump exchanges internal Na⁺ for external K⁺ in approximately a 1.5 ratio^{4,5}. Thus, when Na⁺-loaded/K⁺-depleted erythrocytes are incubated in physiological conditions they tend to recover their original low Na⁺/high K⁺ content. Surprisingly, in erythrocytes from healthy donors the net Na⁺ extrusion/K⁺ influx ratio exceeds the 1.5 ratio predicted for Na⁺, K⁺-pump-mediated fluxes whereas it is similar to this value in erythrocytes from essential hypertensive patients and some of their descendants⁶. We now report that this difference is due to the presence of a Na⁺, K⁺-co-transport system in normal erythrocytes which extrudes both internal Na⁺ and K⁺ and is functionally deficient in erythrocytes of essential hypertensive patients and some of their descendants. No difference in passive Na⁺ permeability could be detected between normotensive and hypertensive subjects.

The different transmembrane pathways involved in net Na⁺ and K⁺ transport in Na⁺-loaded/K⁺-depleted erythrocytes were analysed in the following groups⁶: normotensive and secondary hypertensive subjects devoid of familial hypertension, benign and accelerated essential hypertensive patients and those offspring of hypertensive patients with an abnormal low ratio of Na⁺/K⁺ net fluxes (⊕ normotensives).

The presence of ouabain (0.1 mM) in the incubation medium greatly reduced net Na⁺/K⁺ movements in all subjects studied (Table 1). If we assume that the ouabain-sensitive fraction of the total fluxes corresponds to the exchange of internal Na⁺ for external K⁺ catalysed by the Na⁺, K⁺-pump (see ref. 7 for discussion), three conclusions can be drawn:

First, in erythrocytes of ⊕ normotensives and benign essential hypertensive patients the pump fluxes are 20-40% higher than in normal erythrocytes. A high (Na⁺ + K⁺)ATPase activity has been recently reported for benign essential hypertensives⁸. This increased pump activity could result either from a more rapid rate of cation translocation or from a higher density of pump unities. To test this latter hypothesis we have studied the binding of ³H-ouabain which is known to label the (Na⁺ + K⁺)ATPase specifically. Scatchard analysis of ³H-ouabain binding to erythrocyte membranes indicated the presence of only one class of binding sites, the number of which did not significantly differ between the five groups (Table 1). In contrast, the apparent dissociation constant (K_D) varied from 3.7 × 10⁻⁹ M to 1.3 × 10⁻⁸ M. A correlation was found between the apparent dissociation constant and the pump activation (Fig. 1). It is interesting that a regulatory membrane protein changing the apparent affinity for the ouabain inhibition of the (Na⁺ + K⁺)ATPase has been described recently⁹. This phenomenon certainly merits further investigation.

Second, in all subjects the stoichiometry of the Na⁺, K⁺-pump is very close to that reported previously^{4,5}, that is, ouabain-sensitive Na⁺ efflux/K⁺ influx = -1.5; the minus sign indicates that the vectorial transports of Na⁺ and K⁺ have different signs.

Third, in the presence of 0.1 mM ouabain a net Na⁺ extrusion in an uphill direction against the electrochemical Na⁺ gradient was observed in erythrocytes of normotensive controls (and

Table 1 Characteristics of net Na⁺ and K⁺ transport in erythrocytes from normotensive and hypertensive patients

Flux component	Transport system	Net flux*	Absence of familial hypertension		Familial history of hypertension		
			Normotensive controls	Secondary hypertensives	⊕ Normotensives	Essential hypertensives	Accelerated
			(n = 7)	(n = 4)	(n = 6)	(n = 8)	(n = 6)
Total	All systems	Na ⁺	-3.70 ± 0.24	-3.91 ± 0.68	-3.95 ± 0.87	-3.92 ± 0.81	-2.74 ± 0.39§
		K ⁺	+1.10 ± 0.35	+1.47 ± 0.22	+2.32 ± 0.59§	+2.16 ± 0.45§	+1.55 ± 0.28
		Na ⁺ /K ⁺	-3.59 ± 0.89	-2.67 ± 0.24	-1.72 ± 0.10§	-1.82 ± 0.27§	-1.78 ± 0.09§
Ouabain-sensitive	Na ⁺ , K ⁺ -pump	Na ⁺	-3.20 ± 0.23	-3.35 ± 0.48	-4.64 ± 0.51§	-4.52 ± 0.67§	-3.02 ± 0.48
		K ⁺	+2.08 ± 0.29	+2.13 ± 0.40	+2.87 ± 0.42‡	+2.79 ± 0.43‡	+2.12 ± 0.32
		Na ⁺ /K ⁺	-1.59 ± 0.19	-1.58 ± 0.25	-1.63 ± 0.15	-1.63 ± 0.19	-1.38 ± 0.18
Ouabain-resistant	Non-pumped fluxes	Oub N _s	542 ± 183	540 ±	535 ± 185	637 ± 63	486 ± 119
		Na ⁺	-0.51 ± 0.17	-0.55 ± 0.49	+0.69 ± 0.40	+0.61 ± 0.30	+0.27 ± 0.21
		K ⁺	-0.98 ± 0.15	-0.66 ± 0.20	-0.55 ± 0.26	-0.63 ± 0.24	-0.57 ± 0.27
Ouabain-resistant furosemide-sensitive	Na ⁺ , K ⁺ -co-transport	Na ⁺	-0.52 ± 0.11		-0.10 ± 0.02‡	-0.14 ± 0.18§	
		K ⁺	-0.76 ± 0.27		-0.13 ± 0.08‡	-0.19 ± 0.16§	
		Na ⁺ /K ⁺	+0.73 ± 0.21				
Ouabain- and furosemide-resistant	Passive permeability†	Na ⁺	+0.25 ± 0.05			+0.26 ± 0.06	
			(n = 10)			(n = 10)	
		K ⁺	-0.31 ± 0.17			-0.31 ± 0.12	

The effect of ouabain on the net Na⁺ and K⁺ fluxes was studied by the method previously described⁶ with the following modifications: (1) the PCMBs concentration of the Na⁺-loading solution was reduced to 0.02 mM, (2) 2 mM adenine, 3 mM inosine and 2.9 mM Na⁺ phosphate buffer (pH 7.2 at 37° C) were added to the recovering solution. After recovery, the internal cation content was (mmol per l cells) 55–75 Na⁺ and 30–50 K⁺ and the increase in cell volume was less than 2%, (3) ouabain-sensitive fluxes were calculated by the difference between net Na⁺ and K⁺ fluxes in a Na⁺, K⁺-Ringer solution with and without ouabain 0.1 mM. At hours 0, 1, 2 and 3, two tubes were transferred to 0° C and the cells were washed three times with 150 mM choline Cl, 2.5 mM Tris-phosphate (pH 7.2 at 4° C) and 0.02 mM ouabain at 4° C. Na⁺, K⁺ and haemoglobin were measured in the same sample. To study the effect of furosemide on the ouabain-resistant net Na⁺ and K⁺ fluxes some additional modifications were introduced: (1) the Na⁺-loading solution was (mM) 250 choline Cl, 40 NaCl, 10 KCl, 2.5 Na phosphate (pH 7.2 at 4° C), 1 MgCl₂ and 0.02 PCMBs, (2) the recovering solution was K⁺ free. After recovery the reduction in cell volume was less than 3% and the internal cation content was 20–30 mmol per l cells of both Na⁺ and K⁺. Furosemide-sensitive fluxes were calculated by the difference between net Na⁺ and K⁺ fluxes in a medium containing (mM) 90 choline Cl, 60 NaCl, 2.5 Na phosphate (pH 7.2 at 37° C), 1 MgCl₂, 10 glucose and 0.1 ouabain with and without 1 mM furosemide. At each incubation time three tubes were removed from the incubation bath. Ouabain + furosemide-resistant net Na⁺ fluxes were measured in fresh cells. After removing the plasma and buffy coat, the cells were washed twice with 150 mM choline Cl. They were then incubated at 37° C in three different Na⁺-media containing (mM) 1 MgCl₂, 2.5 Tris-phosphate (pH 7.2 at 37° C), 10 glucose, 0.1 ouabain, 1 furosemide and 0, 75 or 150 NaCl (the isotonicity was maintained with choline Cl). At hours 0, 1 and 2, two tubes were transferred to 0° C and the cells were processed as before. The rate constant of Na⁺ permeability was obtained from the slope relating the ouabain- and furosemide-resistant net Na⁺ fluxes against the external Na⁺ concentration. Ouabain- and furosemide-resistant net K⁺ fluxes were measured in the same PCMBs-treated cells used for measuring furosemide-sensitive fluxes. Ouabain-binding experiments were carried out with washed erythrocytes incubated in (mM) 1 HEPES (pH 7.4), 150 NaCl, 3 ATP and 3 MgCl₂ for 3 h at 37° C with 5 × 10⁻¹⁰ M to 10⁻⁷ M ³H-ouabain (13 Ci mmol⁻¹, NEN). Results were corrected by subtracting the nonspecific binding measured in the additional presence of 10⁻⁶ M unlabelled ouabain. The values given represent mean ± s.d. and *n* denotes the number of subjects in each group. The statistical significance of the data was studied using the non-parametric test of Mann-Whitney.

* Net flux is given in mmol per l cells per h. A minus sign indicates a net cation extrusion, a plus sign a net cation influx. Na⁺/K⁺ values indicate the stoichiometry of Na⁺/K⁺ net fluxes, and Oub N_s the number of ouabain binding sites per cell.

† Rate constant of cation permeability in d⁻¹.

‡ P < 0.01; § < 0.001.

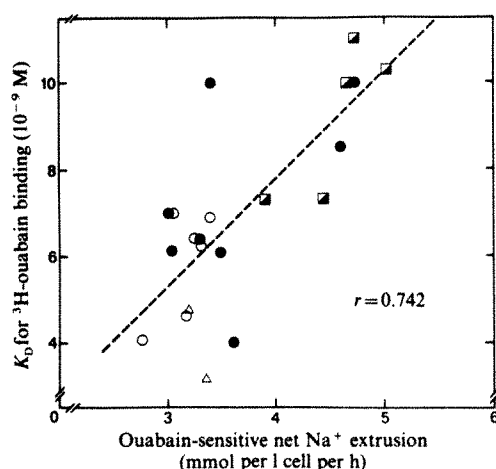


Fig. 1 Relationship between the Na⁺, K⁺-pump activation and the apparent dissociation constant of ouabain binding. ○, normotensive controls; ●, essential hypertensives; △, secondary hypertensives; ■, normotensives born of hypertensive parents.

secondary hypertensives). This confirms previous observations suggesting that the ouabain-resistant fluxes do not simply represent passive permeabilities¹⁰ and that the Na⁺, K⁺-pump is not the only mechanism ensuring a net cell Na⁺ extrusion^{11,12}. It is interesting that no ouabain-resistant net Na⁺ extrusion was observed in erythrocytes from ⊕ normotensives and essential hypertensive patients (Table 1).

In preliminary experiments we found that the pump-independent net Na⁺ extrusion of normal erythrocytes could be inhibited by raising the external K⁺ concentration, by adding 1 mM furosemide or by ATP depletion, thus showing properties similar to the Na⁺, K⁺-co-transport system described in rat¹³, avian¹⁴ and human erythrocytes^{15,16}. In certain experimental conditions, this system may extrude internal Na⁺ in an uphill direction against the electrochemical Na⁺ gradient, using the energy supplied by the downhill K⁺ extrusion across the electrochemical K⁺ gradient. This system was therefore investigated in experiments in which conditions were modified to measure satisfactorily the small ouabain-resistant net Na⁺ and K⁺ fluxes (see Table 1).

In the presence of 0.1 mM ouabain, the addition of 1 mM furosemide to normal erythrocytes loaded with Na⁺ and choline effectively inhibited a net Na⁺ and K⁺ extrusion coupled in a ratio slightly less than +1 (the plus sign indicates that vectorial transport of both ions has the same sign) (Table 1). In contrast,

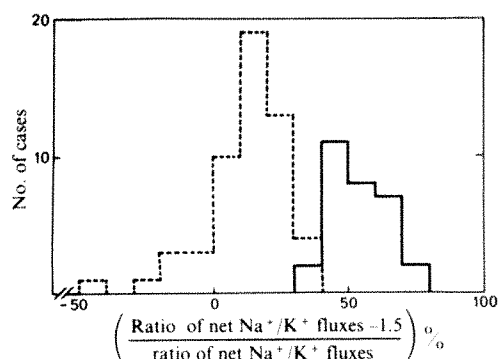


Fig. 2 Histograms of the ratio of total Na^+/K^+ net fluxes represented as the percentage deviation from the Na^+/K^+ -pump stoichiometry. Continuous line represents normotensive controls (30 subjects) and dotted line essential hypertensive patients (54 subjects).

the net Na^+ and K^+ extrusion catalysed by this Na^+/K^+ -co-transport system is markedly reduced in erythrocytes of essential hypertensive patients and \oplus normotensives (Table 1). Although the Na^+/K^+ -co-transport fluxes are smaller than the pump fluxes, the fact that they have a positive stoichiometry as opposed to the negative stoichiometry of the Na^+/K^+ -pump (and of the passive net Na^+ and K^+ fluxes) introduces a marked difference between the stoichiometry of total net fluxes of essential hypertensives and normotensive controls (see Fig. 2).

Net Na^+ and K^+ fluxes in the presence of both ouabain (0.1 mM) and furosemide (1 mM) have the kinetic properties of passive 'leak' fluxes. In fresh red blood cells, the ouabain- and furosemide-resistant net Na^+ influx (1) increases linearly with the external Na^+ concentration, (2) is not modified by external K^+ and (3) becomes zero when the external Na^+ equals the internal Na^+ concentration (in mmol l^{-1} cell). Moreover, these properties do not seem to be modified by incubation with cold 0.02 mM 2,5-*p*-chloromercuribenzenesulphonate (PCMBs). As indicated in Table 1, no difference in the passive Na^+ permeability could be detected between erythrocytes of hypertensive patients and those of normotensive controls. The same is true for the passive K^+ permeability which was only measured in PCMBs-treated cells. Thus, the increase in the ouabain-resistant unidirectional Na^+ influx¹⁷ and Na^+ efflux¹⁸ previously reported in erythrocytes from essential hypertensives, rather than being due to an increased passive Na^+ permeability, might reflect a disorder in a specific transport system such as the ouabain-resistant one-to-one Na^+/K^+ exchange which is activated in erythrocytes from essential hypertensive patients¹⁹. If this were the case, the increased unidirectional Na^+ fluxes would not be related to our observations because the Na^+/K^+ exchange mechanism does not result in net Na^+ fluxes.

The results presented here indicate that the net extrusion of an erythrocyte Na^+ load is accomplished by two different mechanisms, the Na^+/K^+ -pump and Na^+/K^+ -co-transport, which operate against the passive net Na^+ fluxes. Furthermore, they suggest that an inherited defect in the Na^+/K^+ -co-transport system may be genetically associated with essential hypertension. In avian red cells this transport system participates in the regulation of cell volume and is under hormonal control. These observations have led us to formulate the hypothesis that essential hypertension may result from an inefficient hormonal regulation of the extrusion of a cell Na^+ load after an excess Na^+ intake due to a functional disorder of the Na^+/K^+ -co-transport system. Such a process in excitable cells of high surface/volume ratio, such as smooth muscle cells or catecholaminergic neurones, may lead to a temporary or permanent increase in intracellular Na^+ , producing critical changes capable of raising blood pressure^{20,21}. The high Na^+/K^+ -pump activity seen in \oplus normotensives and benign essential hypertensives may therefore represent a compensatory mechanism for extruding a cell Na^+ load and thus preventing severe hypertension in subjects with this genetic abnormality.

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Mechanism of action of dietary fibre in the human colon

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Fibre, more than any other dietary component, affects human large bowel function, causing an increase in stool output, dilution of colonic contents, a faster rate of passage through the gut and changes in the colonic metabolism of minerals, nitrogen and bile acids^{1–6}. (Fibre here refers to 'dietary fibre', which comprises plant cell wall polysaccharides and lignin, and not to 'crude fibre'.) It is thought that these changes are brought about by fibre passing through the gut undigested and holding water within its cellular structure^{7,8}. Although the amount of water taken up *in vitro* varies for different types of fibre^{8–10}, this does not correlate in the expected way with the effects these materials have on colonic function⁹. This is because fibre is extensively degraded in the gut^{2,11}, probably by the colonic microflora^{12,13}. Using a newly developed method modified from ruminant nutrition for isolating bacteria, we have shown that the main component of human faeces is bacteria¹⁴. We show here that the way in which two contrasting types of dietary fibre act in the colon depends on the extent to which they are digested. Cabbage fibre, which is extensively broken down, provides a readily usable substrate for the stimulation of microbial growth, whereas wheat fibre remains largely undigested and retains water in the gut lumen.

Table 1 shows the effect of wheat fibre in six subjects. On the control diet, faecal solids were 55% bacteria, 17% undigested fibre and 24% water-soluble material. With fibre, stool output increased by 102 g per day (127%), of which 19 g was solids and 83 g water. Transit time decreased by 25.1 h and total nitrogen excretion increased from 1.5 to 2.0 g per day. The 19 g per day increase in excretion of solids was accounted for largely by an increase of 11.6 g per day in undigested fibre. Thus, only 36% of the wheat fibre added to the diet was digested in the gut.

If it is assumed that the extra fibre excreted was hydrated to the same extent as the total stool, which was 3.2 g water per g faecal solids, or 76.3% water, this would account for 48.7 g (48%) of the increase in faecal output. The water-holding

capacity of this wheat fibre, when measured *in vitro*, is in excess of 3.2 g water per g material⁹. Similarly, the extra bacteria excreted (2.3 g) would contribute 9.7 g (10%) of the increase at the same hydration. Bacteria are normally about 80% water¹⁵ and, as they comprise the major fraction of faecal solids, are an important water-holding component in the gut. The whole stool was wetter with the added fibre than on the control diet, which suggests that a further 20.5 g (20%) of the increase was water associated with the bacteria and fibre from the control diet. This leaves 23 g (22%) of the increase to be accounted for by water-soluble solids (5.1 g) and water (18.1 g), which may represent an increase in extracellular, or free, water in the stool.

Wheat fibre, therefore, by surviving digestion in the intestine, alters colonic function by holding water and thus increases the bulk of the colonic contents. Transit time is thereby decreased and less water is absorbed from the lumen. Scanning electron microscope studies of bran before and after passage through the gut suggest it retains its cellular structure virtually intact^{16,17}.

The changes seen with cabbage fibre were quite different. Stool output increased by 54.3 g per day (69%), a smaller increase than with wheat fibre but nevertheless highly significant. Transit time fell by 15.8 h and nitrogen excretion increased from 1.5 g to 2.1 g per day. The increase in stool output comprised 8.5 g per day solids and 45.8 g water. Of the solids only 1.6 g was undigested fibre, indicating that on average 92% of the cabbage fibre had been digested. The main increase was in bacterial solids (4.8 g) which, at the hydration of the stool (74.6%), accounts for 18.9 g (35%) of the increase in stool weight. The faeces were again better hydrated on the cabbage fibre diet than during the control, presumably because the increased bacterial mass stimulated faster transit and led to less water absorption from luminal contents by the large bowel mucosa. These changes in hydration account for a further 17.5 g (32%) of the stool output, the extra fibre excreted adds 6.3 g (12%), and the rest, 11.6 g (21%), is associated with the soluble component.

Cabbage fibre, therefore, influences colonic function through its stimulation of microbial growth. Analysis of the bacterial fraction for nitrogen (for three subjects) shows that 0.42 g of the 0.67 g per day (63%) increase in nitrogen excretion is associated with this fraction, whereas with wheat fibre (four subjects) only 0.18 g of the 0.53 g (34%) increase is in the bacteria¹⁸. The rest of the nitrogen is presumably present in the undigested cell-wall material.

Fibre provides a ready source of available carbohydrate to the microflora of the human colon, as it does in the rumen. On the

Table 2 Changes in faecal composition (g per day \pm s.e.m.) and transit time (h) with cabbage fibre

	Control diet	+Cabbage
Mean daily stool weight	88.2 \pm 9.8	142.5 \pm 16.1*
% Moisture	69.4 \pm 1.9	74.6 \pm 2.0†
Total solids	26.1 \pm 1.2	34.6 \pm 5.7*
Total nitrogen	1.5 \pm 0.1	2.1 \pm 0.1†
Transit time	79.5 \pm 10.4	63.7 \pm 8.0‡
Composition of solids (g per day)		
Neutral detergent fibre	4.1 \pm 0.2	5.7 \pm 0.4*
Water soluble	6.0 \pm 0.5	8.7 \pm 0.7†
Bacteria	14.5 \pm 1.1	19.3 \pm 0.8†

Six healthy male subjects, three of whom also took the bran, undertook the same protocol as outlined in Table 1. Instead of wheat fibre, 18.3 g per day of cabbage fibre was added to the diet.

* $P < 0.001$; † $P < 0.01$; ‡ $P < 0.05$.

basis of calculations from rumen physiology, the digestion of 16.4 g per day of fibre from cabbage would be expected to produce 0.44 g per day bacterial nitrogen, which approximates closely to the findings in this study (0.42 g per day).

Two distinct mechanisms thus emerge whereby fibre affects the human colon. We believe that the stimulation of microbial growth is the more usual one in man because very little fibre survives digestion by the bacteria when sources such as apple, carrot, guar (unpublished observations), pectin¹¹ or mixed diets^{2,21} are fed. Wheat fibre, and possibly cereals in general, may prove to be the exception as they have small cells with highly lignified cell walls which resist digestion.

The increase in excretion of bile acids, neutral sterols, nitrogen, fat, minerals and electrolytes which have been noted in other studies when fibre is fed, have been ascribed to the physical effect of the fibre itself^{2,3,19-23}. Because little fibre is excreted in the faeces, other explanations for these changes must be sought, perhaps taking account of the increase in bacterial mass.

On the control diet, both stool output and transit through the gut were significantly correlated in this study with faecal bacterial mass ($r = 0.84$ and -0.80 respectively). If, as has been suggested²⁴, colonic disease is more common in people who have small stool outputs and slow transit, the control of colonic microflora will be important in determining disease susceptibility in individuals. The type, amount and digestibility of fibre in the diet will, in turn, make a significant contribution to this.

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Table 1 Changes in faecal composition (g per day \pm s.e.m.) and gut transit time (h) with wheat fibre

	Control diet	+ Wheat fibre
Mean daily stool weight	95.5 \pm 8.7	197.0 \pm 13.4*
% Moisture	71.1 \pm 1.5	76.3 \pm 1.2†
Total solids	27.0 \pm 1.2	46.0 \pm 1.1*
Total nitrogen	1.5 \pm 0.1	2.0 \pm 0.1†
Transit time	67.8 \pm 10.1	42.7 \pm 2.7‡
Composition of solids (g per day)		
Neutral detergent fibre	4.6 \pm 0.4	16.2 \pm 0.3†
Water soluble	6.5 \pm 0.5	9.2 \pm 0.4*
Bacteria	15.0 \pm 0.7	17.3 \pm 1.0‡

Six healthy male subjects, aged 22-38 yr, were given a controlled diet for 3 weeks consisting of everyday foods of overall daily composition: 385 g carbohydrate, 85 g protein, 108 g fat and 22 g dietary fibre. For a further 3-week period 18 g dietary fibre as bran was added to the diet. Further details of the study protocol and methods of preparation of the fibre are recorded elsewhere¹. Transit was measured by the continuous marker method²⁵. Faeces were collected throughout, and for each subject those from the last 7 days of each dietary period were pooled and freeze-dried. Daily excretion of fibre, as neutral detergent fibre²⁶, nitrogen by the Kjeldahl technique, bacterial mass and the water-soluble component of faeces were measured¹⁴.

* $P < 0.001$; † $P < 0.01$; ‡ $P < 0.05$.

BOOK REVIEWS

Phylogenetic hypotheses

R. D. Martin

THIRTEEN years have elapsed since the late Willi Hennig's influential textbook *Phylogenetic Systematics*, translated from the German manuscript by Dwight Davis and Zangerl, was originally published in 1966. Republication, with the addition of a brief but useful introduction by three disciples, comes at a time when Hennig's major contribution to the science of phylogenetic reconstruction has more-or-less achieved the widespread recognition that it deserves. This is also a particularly suitable time to take stock of Hennig's approach. Although *Phylogenetic Systematics* is set out with admirable clarity, considerable controversy has accompanied the dissemination and practical application of Hennig's concepts. The issues at stake can only be clearly appreciated by taking a step omitted by Hennig and many others in the field — namely, that of considering separately phylogenetic reconstruction and classification. For the first, Hennig has unquestionably introduced greater scientific precision, while for the second he has arguably generated further confusion.

The attempt to reconstruct phylogenetic history is, or should be, a scientific undertaking involving the identification of past evolutionary relationships ultimately embodied in the process of speciation. One of Hennig's most important innovations here lies in his explicit statement that mere assessment of degrees of morphological (or

Phylogenetic Systematics. By W. Hennig. Pp. 263. (University of Illinois Press: London, 1979.) £12.

other) similarity does *not* provide an adequate basis for accurate phylogenetic reconstruction. Any given group of organisms can be theoretically traced to an original stem species with a certain array of initial (plesiomorphous) characters, and retention of any of these characters as shared similarities among descendant species provides no information about subsequent branching in the evolutionary tree. Only derived (apomorphous) characters developed at some later stage and retained in certain descendants can be used as indicators of phylogenetic relationships within the group. Hennig provides a number of empirical guidelines for the differential assessment of shared similarities (plesiomorphous; apomorphous; convergent) in phylogenetic reconstruction, and concepts such as those of the character transformation series and of the sister group are of considerable heuristic value. However, as Hennig himself recognises, everything in the end boils down to a scientific estimation of probabilities. Thus, the accuracy of phylogenetic reconstruction depends on the validity of the theoretical principles used (hence the value of Hennig's contribution) and upon the diversity of the

characters examined.

Unfortunately, Hennig simultaneously maintains that classifications should be directly based upon the evolutionary branching pattern and the dates of divergence inferred. This controversial tenet has proved to have at least two great drawbacks. Firstly, there is no compelling reason to assume that classifications should only reflect inferences regarding the positions and timing of branching points in phylogenetic trees. Secondly, since every phylogenetic reconstruction represents a hypothesis based on estimation of probabilities, Hennig's prescription inevitably leads to a proliferation of alternative classifications and instability of higher taxonomic categories. Since classifications provide, among other things, the terms we use to discuss living organisms, taxonomic instability is associated with linguistic instability. This being the case, any recommendation to read Hennig's excellent logical dissection of the process of phylogenetic reconstruction must be tempered with the warning that classification (which inevitably involves numerous arbitrary decisions) will not necessarily benefit from too close an association with the phylogenetic hypotheses of individuals.

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For the petroleum geologist

R.C. Selley

Petroleum Geochemistry and Geology. By J.M. Hunt. Pp.617. (Freeman: San Francisco and London, 1979.) £16.95.

THE application of chemistry to the study of oil generation, migration and maturation has a long history. It is only within the last decade, however, that geochemical exploration techniques have become an accepted tool in the oil industry. Dr Hunt has played a seminal role in these advances both in industry, with Exxon,

and latterly at Woods Hole. This book thus merits the attention of every petroleum geologist. It consists of twelve chapters, arranged in four parts: Introduction, Origin and Migration, Habitat, and Applications.

The Introduction begins with a short historical review and continues with an account of the early formation of life on Earth, photosynthesis, the carbon cycle and the preservation of organic carbon in sedimentary rocks. This is followed by a chapter on the chemistry of hydrocarbons, both those which occur naturally and those produced by refineries.

Part II, Origin and Migration, begins with a cursory review of the abiogenic theories of hydrocarbon formation before launching into an account of the chemistry of plant and animal tissues and the

diagenetic changes which they undergo on burial. The temperature required for oil generation is discussed from both theoretical and observational points of view. Gas generation is treated in a separate chapter. The final chapter of this section deals with the last great mystery of oil, namely the magic of primary migration from the source rock to the carrier beds.

Part III, Habitat, opens with an account of source rocks, discussing the oil and gas generating potential of the various types of kerogen, and reviewing the various palaeothermometers available from maturation studies. This is followed by a chapter on the variation and distribution of hydrocarbons in reservoirs, with respect to depth, age and degradation.

Part IV, Applications, shows how the data and concepts previously presented

may be used in hydrocarbon exploration. This begins with a review of surface manifestations of oil, both the visible seeps of oil and gas onshore and offshore, and surface geochemical prospecting. A chapter on subsurface prospecting follows, describing the analysis and interpretation of cuttings gas as well as ways in which source rocks may be studied to determine their oil or gas potential, and their degree of maturity.

The next chapter shows how crude oils may be correlated with their source beds, and the text concludes with a short account of geochemical prospect evaluation. Finally, there is a glossary, forty pages of references and author and subject indexes.

Inevitably, this book invites comparison with Tissot and Welte's recent book, *Petroleum Formation and Occurrence* (Springer: Berlin, Heidelberg and New York, 1978), on the same topic. They both cover the same subject matter and both draw freely (and with due acknowledgement) on each other's work. Both books are excellent and the only simple comparison that may be drawn is that Hunt's

book perhaps emphasises geology more than chemistry, while for Tissot and Welte's book the converse is true. Certainly, this reviewer, who is only a geologist, finds Hunt's book the easier to read.

The arrangement of the vast amount of material in a book of this kind presents many problems but could perhaps have been improved. For example, discussion of source rocks should perhaps precede an account of the emigration of hydrocarbons from them. Sections dealing with palaeo-temperature analysis could perhaps have been grouped together, and their relative merits and limitations contrasted, instead of their being distributed through several chapters of the book. Since oil and gas formation is so intimately related it seems an unnecessary distinction to discuss their genesis in separate chapters.

To counteract these comments, however, it must be stated that the book is well written, well researched and pleasantly produced. All the line illustrations have been specially prepared for this work and

each part begins with a half-tone plate. A particularly helpful feature is the inclusion of a summary and list of supplementary reading at the end of each chapter.

The wide range of case histories discussed is impressive. It is unusual to find a geologist of the USA who is familiar with, or at least prepared to quote, data from outside North America. This reviewer could spot only two important omissions. There was no reference to Porfir'ev's paper on the inorganic origin of oil (*Bull. Am. Ass. Petrol. Geol.* 58, 3-33, 1974). This is probably the most accessible exposition of the Russian igneous oil theory for readers in the free world. Several papers related to the correlation of vitrinite reflectivity and electron spin resonance are also omitted.

These are, however, minor blemishes on a major textbook. No petroleum geologist should be without a copy of *Petroleum Geochemistry and Geology*. □

R.C. Selley is Reader in Petroleum Geology at Imperial College, University of London, UK.

Plankton research

C.M. Yonge

Zoogeography and Diversity in Plankton. Edited by S. van der Spoel and A.C. Pierrot-Bults. Pp.410. (Edward Arnold: Maidenhead, 1979.) £37.50.

THIS most attractive book is a revelation of the extent and cohesion of plankton research. It appears at about the centenary of the initial work by Hensen which led, at the beginning of the century, to the formulation by the International Council for the Exploration of the Sea of a programme involving the quantitative study of plankton in relation to hydrography on the one hand and fisheries on the other. These first collections and counting of plankton have gradually spread over the face of globe, with whaling investigations in the Southern Ocean and the International Indian Ocean Expedition extending knowledge into these previously little known areas.

Problems of distribution and of speciation, the dominant themes in this book, are vast and perplexing. In contrast to the terrestrial habitat, that of marine plankton is three-dimensional and continuous. There are notable distinctions between neritic (coastal) and oceanic populations, with latitudinal divisions largely temperature based and different biotopes in depth represented by epipelagic, mesopelagic and bathypelagic plankton. Distinctions between habitats are often best defined by the resident 'indicator' species, most frequently

chaetognaths. The *Challenger* studies of Murray and Renard on the deposition of planktonic skeletons on the ocean floor are the basis of modern work on the history of ocean basins and provide important evidence concerning continental drift.

An adequately authoritative survey of this immense field demands specialist treatment and most exacting editorial standards. Both are supplied by the contributions of fourteen authors from seven countries, most competently

organised by S. van der Spoel and A.C. Pierrot-Bults of the Institute of Taxonomic Zoology, University of Amsterdam. Clearly drawn figures and an extensive literature list complete the attractions of a book which presents information essential to all interested in the full extent of biological enquiry. It makes immediate appeal to the scientific imagination. □

Sir Maurice Yonge is Honorary Fellow in Zoology at the University of Edinburgh, UK.

Light physics

P. W. Hawkes

Optoelectronic Devices and Optical Imaging Techniques. By D. A. Ross. Pp. 137. (Macmillan: London, 1979.) Hardback £12; paperback £4.95.

THIS clear, simple introduction to a range of devices that convert electrical energy into light or generate such energy from light will appeal to a wide student audience, particularly to those who need to acquire only a smattering of the physics involved before exploring the many applications. The text is orientated towards specific devices, chapters being devoted to LEDs, photoconductors, photodiodes and phototransistors, solar cells and laser diodes; two other chapters are concerned with noise in such devices and in optical recording media, first film and then CCDs. All these are described in easy, uncomplicated language and illustrated

with uncluttered line diagrams and a photograph showing CCD-aided decapitation of carrots! I was a little startled to encounter, for the first time for many years, the jovial, avuncular style of older school textbooks; a line diagram is described as "An artist's impression of a photon", we are told that "It would be a minor disaster to be forced to abandon Fourier analysis . . .", but isolated examples cannot properly convey the flavour which nonetheless pervades the book.

The publishers have sensibly also brought out the book in paperback. Libraries will of course be more interested in the cased edition, but the paperback version is good value at £4.95; it should sell well, not only to students but to others, since plenty of people are interested in understanding these devices by which they are surrounded. □

P.W. Hawkes is Maître de Recherches in the Laboratoire d'Optique Electronique du CNRS, Toulouse, France.

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Edited by

Professor S. Gregory

Department of Geography, University of Sheffield

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*Department of Geography, University of Leeds,
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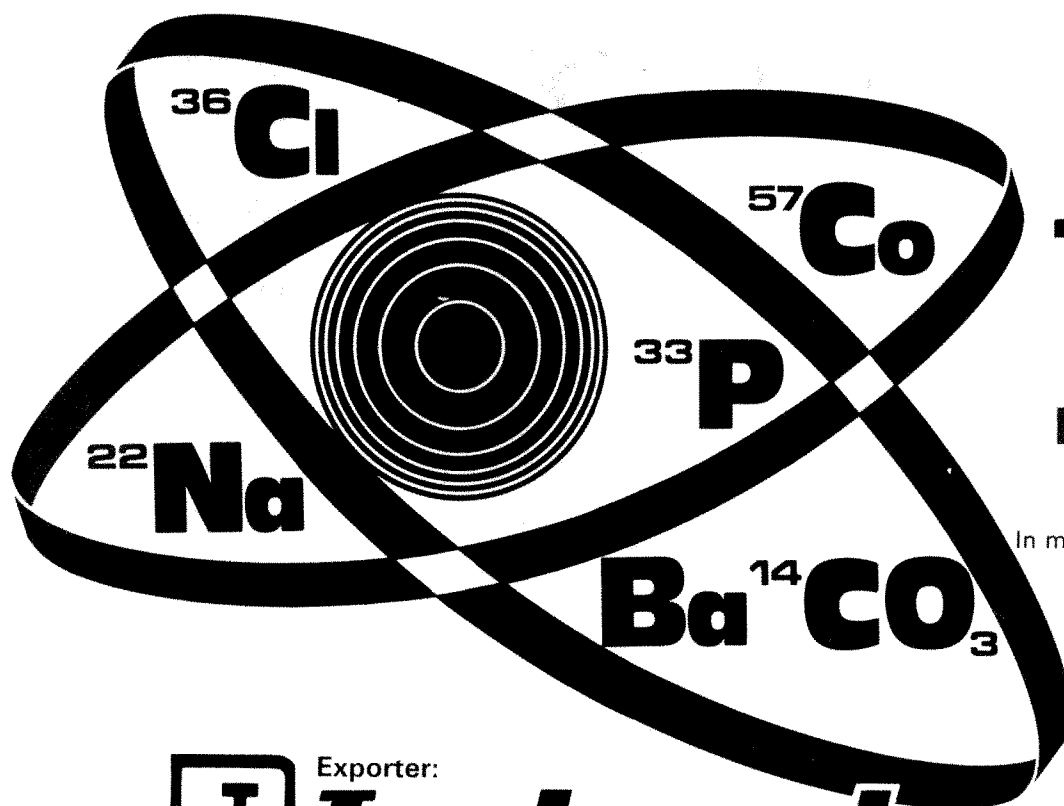
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Transfer RNA

J.P. Goddard

Transfer RNA: Structure, Properties, and Recognition. Edited by P.R. Schimmel, D. Söll, and J.N. Abelson. Pp.577. (Cold Spring Harbor Laboratory: New York, 1979.) \$60.

NEARLY a quarter of a century has passed since the existence of the small adapter molecules known as transfer RNA was predicted. Their subsequent study has been motivated by their properties. They are relatively small and their central role in protein synthesis involves many specific interactions with proteins and nucleic acids. Furthermore they have since been strongly implicated in regulation of gene expression. The extent of this study is reflected in the fact that two volumes — the second is on biological aspects — were found necessary to fulfil the aim of summarising the knowledge of tRNA up to about the end of 1978.

The means to that end is, as in previous Cold Spring Harbor monographs, a series of articles by specialists in a particular aspect of tRNA who participated in the Transfer RNA Meeting at Cold Spring Harbor in August 1978. These articles have been supplemented by four appendices — on tRNA sequences and a proposed numbering scheme, on modified nucleosides and their chromatographic mobilities, and the characteristics of aminoacyl-tRNA synthetases. This first volume contains some thirty-one articles covering aspects of primary structure and isolation, crystal structure analysis, solution studies, aminoacyl-tRNA synthetases, synthetase recognition, and interaction with the ribosome.

Unlike several of its predecessors, this monograph contains no general review. My immediate reaction to this omission was to praise the economy of the editors in not adding to the several reviews published in the last couple of years. However, a non-specialist seeking in this volume a thumbnail sketch to tRNA research will be disappointed. A short article expanding the contents of the brief preface by putting each article in its context and perhaps citing other recent reviews would have added less than one per cent to the volume's bulk and price but would have increased its value both to new workers in the field and to scientists from other specialities.

This lack is compensated to a large extent by articles which have extensive bibliographies. This is particularly true of the articles beginning each section which are written by the session chairmen at the meeting who refer to articles later in their section. The invited contributors of articles have usually endeavoured to present not only their own work but also that of other

workers in the field. As the writers are, in the main, reporting topics on which they are among the leading experts, many quite reasonably present reviews of their past few years' work interspersed with that of others. This occasionally leads to a one-sided interpretation in areas where there is currently disagreement, e.g. the structure of the *E. coli* ribosome. However, the volume is generally notable for the generosity and objectiveness of the writers in reporting views and ideas contrary to their own. These virtues are not purchased at the cost of dull uniformity. Each article retains its individuality and yet the volume forms a coherent unit.

In the absence of a moratorium, it is fortunately not possible to produce "the

definitive reference work on transfer RNA". Even the proposed numbering system of nucleosides in tRNAs (Appendix IA), so eminently reasonable a year ago, looks strangely dated in the light of the structure of the tRNAs in our own mitochondria. However, this volume is the best we can hope for — a summary of the major results of past tRNA research with hints of future solutions to outstanding problems. It should prove stimulating reading to all those working in tRNA research, and those working in other aspects of gene expression will find many articles of value. □

J. P. Goddard is a Lecturer in Biochemistry at the University of Glasgow, UK.

Unifying the forces of nature

Malcolm MacCallum

Elie Cartan and Albert Einstein: Letters on Absolute Parallelism, 1929-32. Edited by R. Debever. Translated by J. Ritter and J. Leroy. Pp. 233. (Princeton University Press: Princeton, New Jersey, and Guildford, UK, 1979.) £11.40.

I FOUND these 38 letters (and one postcard) an enjoyable and interesting read, but it is difficult to discern the intended readership, and so I do not know to whom I could recommend the book. The reader needs to be polyglot. Einstein wrote in German, Cartan in French. Both are translated, Ritter presumably translating Einstein's letters and Leroy those of Cartan. The preface and notes by the editor, the well-known Belgian relativist, Robert Debever, are, however, given only in French. The translations are passable, though Leroy's seems to show that English is not his mother tongue. The reader also needs a good knowledge of the background, and the energy to read the references, as the book is not self-contained. The editor even omits one manuscript note because a slightly modified version was published, and he gives only bibliographical details of most of the printed material the correspondents exchanged.

The letters cover three main topics. The first is the writing of a historical note on spaces with absolute parallelism which Cartan drafted in only two weeks in May 1929. It appeared, accompanying Einstein's paper on his attempt at a unified field theory using such spaces, in 1930; Einstein had formulated his ideas in ignorance of the mathematical literature. The second, covered by 26 letters between 3 December 1929 and 17 February 1930, deals with alternative systems of field equations in such spaces, and qualitative features of such systems of partial

differential equations linear in first derivatives. Cartan showed there was another set of equations with the same mathematical properties as Einstein's (involuntary character, determinacy of the Cauchy problem, and arbitrariness of initial conditions), and Einstein gave reasons for thinking them less physically satisfactory. This discussion gradually narrowed to the third subject, the question of whether one could define the 'strength' of a set of such equations by counting freely specifiable functions of r variables for every r up to some maximum. Cartan argued that only the maximal r gave a meaningful result. The two authors never agreed, even in the seven later letters exchanged in 1931 and 1932. By 1932 Einstein had abandoned distant parallelism for the Einstein-Mayer theory, and the only later outcome is the much modified idea of 'strength' used in *The Meaning of Relativity* (fifth edition, Appendix II) which Debever does not cite.

What use, then, is the book? The modern researcher, seeking a unified field theory, will find little of direct relevance to present efforts in this direction. The professional historian of science would probably prefer to consult the original manuscripts. The general reader may be put off by the effort required of him. If he is not, then he may be rewarded in several ways. There are interesting personal sidelights on the characters. The speed of the mail, and the authors' energy (especially as Einstein was 50 and Cartan 60) must be admired. There is much to learn from Einstein's attitude to physical problems and from Cartan's clear and careful mathematical expositions, and some reassurance for lesser mortals in their occasional mistakes. Principally, one can understand how two great minds worked in developing an (albeit unsuccessful) attempt at one of the greatest problems in physics, the unification of the forces of nature. □

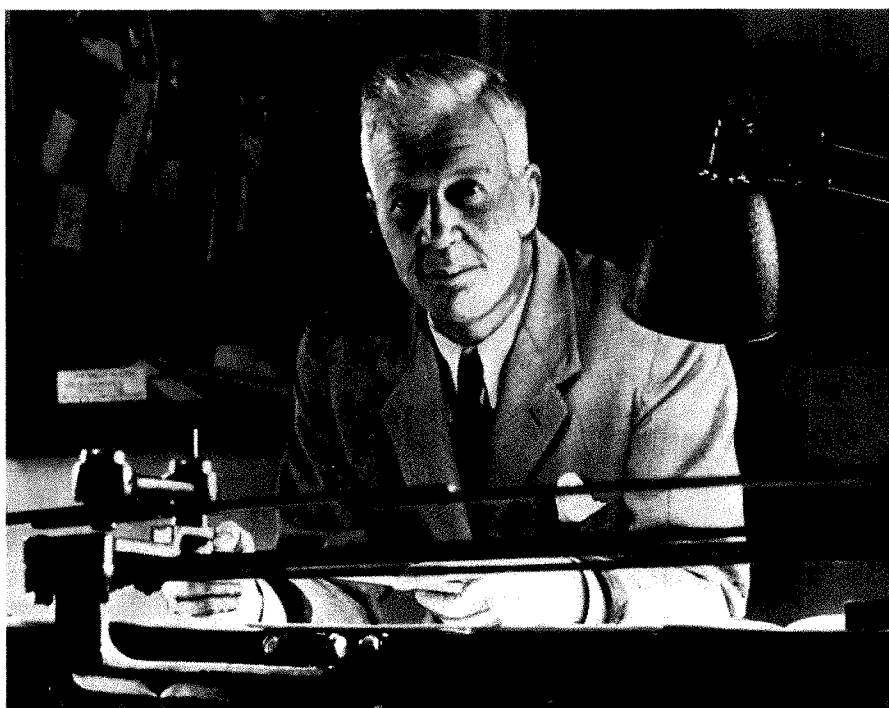
Malcolm MacCallum is a Lecturer in Applied Mathematics at Queen Mary College, University of London, UK.

OBITUARY

Sir Barnes Wallis — 1887-1979

I FIRST heard of Wallis in 1920, when the airship R 80, for the design of which he was responsible, began its trials. In retrospect, this was the most successful rigid airship designed in this country but the Air Staff decided that they had no use for it and it flew for the last time on 20 September 1921. As a result of this decision, Wallis was forced to leave the world of design in which he had already, at the age of 34, proved himself a master. He took up teaching in Switzerland and began his courtship of the lady who was to become his wife. In 1923 however a new association with rigid airship design began. Another remarkable man, Commander (and ultimately Sir) Dennistoun Burney, had been at work during Wallis's absence abroad, gaining support for his airship project. The result of Burney's persistence was the creation of the Airship Guarantee Company, a Vickers subsidiary headed by himself with headquarters at Howden in Yorkshire, and the renaissance of the Royal Airship Works at Cardington. Wallis became the chief designer at Howden and at Cardington the design team was led by V.C. Richmond. The Air Ministry gave the Airship Guarantee Company the task of building R 100 and the Royal Airship Works the task of building R 101.

In 1924 work began in earnest on both designs. In outline they were similar, each five times as long as its greatest diameter, with that diameter at 40% of the length from the nose. They were both to hold 5,000,000 cu.ft. of hydrogen. In 1924 there also began an absurd segregation of the two design teams. I was conscious of it almost from my first day at Cardington, in July 1924. That a tremendous competition should begin in that year was inevitable, and indeed desirable, but that each team should find itself with a boundary wall round it, and have no opportunities to compare notes and progress, was absurd and damaging: each team would have benefited by knowledge of the ideas and progress of the other. Where the blame for this situation, which rapidly developed and was sustained to the end — the end of R 101 — should lie I am uncertain. Wallis, already a successful airship designer, highly individualistic, formidable in his belief — not unjustifiable — in his own ideas, was almost the antithesis of Richmond, whose experience had been with non-rigid airships and whose merits were rather those of a manager and a talent-spotter than of an aircraft designer. Each was scornful of the other's efforts. In



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my five years at Cardington I do not think they ever met. But the long-range animosity — it was no less — was fostered by others, and if Richmond was no genius as Wallis was, he nevertheless had an *eminence grise*, Flt Lieut. (later Sqdn Leader) F.M. Rope at his elbow whom, had Wallis ever met him, he would have had to admit to his own extraordinary intellectual level.

In R 100 Wallis, I believe for the first time, exhibited his passion for helices. The main booms of the heavy triangular section girders were made by rivetting duralumin strip on itself to produce a tube resembling a paper drinking straw. Wallis designed the special rivetting machine for the job. The wiring conveying the lift of the gasbags to the joints of the structure was a system of helices — or, more accurately, geodetics — in which the mesh became finer as the height from the bottom of the ship, and hence the gas pressure, increased. The affection for the helix, which in Wallis's hands produced elegant solutions to structural problems, manifested itself even more dramatically in his two most famous aeroplanes, the Wellesley and the Wellington.

Before the splendid flight of R 100 across the Atlantic and back in the summer of 1930, Wallis had been persuaded by Sir Robert McLean, the managing director of Vickers, to leave Howden and join the

aircraft design side of Vickers. His first appointment was at the Supermarine works at Southampton where he did not cooperate successfully with another remarkable man, R.J. Mitchell, the designer of the Spitfire. After a few months he was moved to Weybridge to work alongside still another great man, R.K. Pierson, famous for a whole series of aircraft, including the Vimy which made the first transatlantic flight in 1919. They were both ranked as Chief Designer.

The association of Pierson and Wallis was a complete success. Pierson was a big man, in physique and in character: soon Wallis, four years the elder, whom at this time I began really to know, called him Uncle Rex, which I think defined Pierson's relations with the major personality so suddenly thrown into close association with him.

While Wallis was back at Howden during the preparations for R 100's Atlantic venture, he was not allowed by McLean to fly in her, which seems a little hard. But by the time he had left Howden for Southampton, Wallis indeed had already begun to doubt the future of the airship in the face of the dramatic progress of the aeroplane, so perhaps he did not feel the deprivation too deeply. He was soon back at Weybridge thinking about aeroplanes and developing the system of geodetic construction.

At that time I was working on criteria defining the stiffness characteristics of aeroplane wings necessary for avoiding wing flutter and loss of aileron control due to wing twisting. Wallis came to see me and said, in effect, "Tell me how stiff to make my Wellesley wing and I will make it that stiff and no more". I told him and he did indeed achieve the minima I prescribed with a structure which was extremely light. The structure weight he achieved was well below what was being achieved in wings of more conventional construction.

I have never been convinced that Wallis's geodetic system was intrinsically superior to more conventional systems. It was superior in practice because he designed extremely efficiently to the prescribed limits. I have no recollection of any other designer of those days seeking at the outset of his design to discover the stiffness limits to which he should design. Even if I do dare to doubt the intrinsic superiority of geodetic construction however, I certainly do not deny its elegance. It had moreover a tremendous advantage in battle. The fact that strength and stiffness were, so to speak, distributed in what was almost a skin of geodesic mesh meant that the structure had few weak points, and gunfire from the ground and from the air might knock great holes in the structure without wrecking it. So triumphant was it that one might well ask why it did not persist as a basic system for aircraft construction. The answer is that the whole trend of design was away from frameworks covered with fabric and towards all metal monocoque construction. That is why the Wellesley and the Wellington were never emulated, even by Wallis himself.

The Wallis I knew was never the arrogant

prickly person we of the R 101 design team were led to visualise. Certainly his was a strong character, certainly he was firm in his views, but equally certainly he knew his limits and did not hesitate to seek help in areas beyond them — as when he sought my aid on wing stiffness — and I am sure he sought Pierson's aid, particularly on the aerodynamic side of his designs. He was indeed sometimes critical of others working in his own areas of expertise, but who is not? I always found him supremely articulate, subtly persuasive and invariably polite, all in a voice it was a pleasure to listen to. I found him, too, appreciative of the work of others. I recall that it was with some trepidation that I took him, at his request, to Hucclecote to see the drawings of the Gloster E 28/39 which Carter was designing for the first flight of the Whittle jet engine. I thought there might be questions of the "Why didn't you do it this way?" kind. There were not. Carter was completely open — he displayed all his ideas and Wallis had nothing but commendation then and afterwards.

I had no contact with Wallis on the Mohne and Eder dambusting bomb nor its massive successor. But I was in touch, as an occasional visitor to Weybridge, with his ideas on the swing wing or variable geometry aeroplane and bitterly disappointed at the lack of support for his ideas. The swing wing has been with us for some years now, but had it been adopted in practice when Wallis showed the way this country would have had a commanding technological lead in a new field: once again, we threw away a golden opportunity.

In his office on the old Brooklands track, and after his retirement from

Vickers in his home, he always worked out his ideas himself on his drawing board. Obviously, on his great projects, he directed the work of others. But the intricacies of joints in geodetic meshes, the details of the pivot of a swing wing, were elucidated on his own board. It was the same to the end: when I last saw him in his house at Effingham he was working on his board on very high speed vehicles indeed. I can only hope his efforts to bring these last visions to reality will not be wasted.

I have made no attempt in these notes to chart the long life of Barnes Neville Wallis, from his birth on 26 September 1887, to his death on 30 October 1979. The fullest account, up till 1970, is Mr Morpurgo's biography¹. I do not agree with quite all of Mr Morpurgo's judgements — nor indeed with quite all of those he attributes to Wallis — but factually it is a vivid and accurate account of a great man's life and I hope the biographer will add a chapter to complete the story. In that book we can read the true account of how Wallis was awarded by the Royal Commission on Awards to Inventors £10,000 for his dambusting bomb, and how he applied that sum to the creation of a foundation at his old school, Christ's Hospital — of which he was Treasurer from 1957 to 1970. This splendid act illumines facets of Wallis's character with which the world in which he became famous was unfamiliar — his loyalty, his generosity, indeed his unselfishness. In total he was a great and good man. We who knew him were greatly privileged.

Kings Norton

¹ Morpurgo, J.E. *Barnes Wallis: A Biography*. (Longman, London 1972).

Wolfgang Yourgrau

WOLFGANG H.J. YOURGRAU died suddenly at his home in Denver, Colorado, USA, on 18 July 1979, at the age of 70, shortly after returning home from an extended tour of lecturing and research in Europe. His passing marks the disappearance from the world of physics and the philosophy of science of a colourful scholar and humanist, whose diverse talents and activities have left enduring imprints far outside the field of his speciality.

Wolfgang Yourgrau was born on 16 November 1908 near Berlin of a Belgian father and a German-Jewish mother. He attended the Werner-Siemens Realgymnasium in Berlin and subsequently studied theoretical physics, mathematics, and biology at the local von Humboldt University, at a time when celebrated physicists such as von Laue, Einstein, and Schrödinger graced its Faculty. Serving first as a tutor in natural philosophy and then as an assistant to Schrödinger, he earned his Dr phil. *magna cum laude* in

1932, the terminal year of the Weimar Republic.

The next year saw the ascendancy to power of the Nazis, and Yourgrau, who got their attention as an organizer of the S.A.P. (an offshoot of the German Social Democratic Party), fled Germany after being severely beaten up by the Storm Troopers. In exile, he remained on the move while lecturing on the evils of fascism in Latvia, Poland, and other European countries, until he was allowed to enter Palestine, then a British mandate, as a political refugee.

Appointed a lecturer with the educational and cultural division of the *Histadrut* (Jewish Federation of Labor), he travelled widely inside Palestine and became drawn into discussions on the intractable problems of this territory. But Nazism, its devastation of the cultural values in which his family had been rooted for centuries, and then the daily progress of World War 2 remained foremost in his mind. In the Spring of 1942 he acquired from the British authorities a license to publish *Orient*, an independent German-

language weekly, with himself as Editor-in-Chief and Arnold Zweig, the renowned novelist-dramatist and a fellow exile, as co-publisher. Under Yourgrau's direction, *Orient* — which has lately become the subject of detailed analyses by some literary historians — declared war "on every fascist movement, every attempt to restrict the right to free expression of opinion . . ." But it was a subsidiary point on the programme of *Orient*, touching on the complex internal politics of Palestine, which made the journal a centre of intense controversy. Boycotts and threats against businesses connected with *Orient* and finally destruction of the premises of its fourth printer caused the financially plagued journal to close down for good in 1943, one year after its inception.

Yourgrau lost no time in assuming a more active role in the war against the Axis Powers, which was then increasingly being fought in the Mediterranean theatre. At first, working from an office of the Palestine Information Office, this only meant preparing the news bulletins in

German that emanated daily from Jerusalem Radio. But after the American Office of Strategic Services established its Middle Eastern Headquarters in Cairo, Yourgrau also was recruited to serve with that organization, vitally assisting it in its planning of some intelligence operations behind enemy lines, details of which are only now being declassified.

At War's end Yourgrau redirected his phenomenal energy into the resumption of his academic career and the pursuit of his pre-empted research. He became Head of the Department of Logic and Scientific Method at the School of Higher Studies in Jerusalem and subsequently Acting Dean of its Faculty of Arts and Sciences. At the request of the Colonial Office he went to Cyprus in 1948 to determine whether a branch of the University of London should be opened on that island. ("Under no circumstances!" his report concluded after 4 months of investigation.) In the same year Yourgrau emigrated to South Africa, having previously married South African-born Thella Garber. For a decade he taught and continued his writing and research at the Universities of Cape Town, Witwatersrand, and Natal.

In 1959 he moved to the United States, first to accept the position of Research Professor at the Minnesota Centre for the Philosophy of Science, and then to become Chairman of the Department of History of Science at Smith College in Northampton, Mass. In 1963 he accepted a permanent position as Professor of History and Philosophy of Science at the University of Denver.

The scope of Yourgrau's publications was prodigious. Ranging from the political editorials in *Orient* to papers on general relativity, they made him known to an exceptionally large spectrum of scholars, a fact that is attested to by the scheduled appearance in 1981 of a memorial volume of essays in his honour written by a diverse and distinguished panel of more than 30 of his academic colleagues. Although most of his papers are devoted to problems in theoretical physics, a large fraction deals with philosophical issues, while some others treat matters of a biographical, literary, or political nature. Of the many books he co-authored or co-edited, *Variational Principles in Dynamics and Quantum Theory* and *A Treatise on Irreversible and Statistical Thermodynamics* are perhaps the best known. In 1969 he founded, with Henry Margenau of Yale University, the international journal *Foundations of Physics*, which he co-edited until his death. He was the recipient of numerous distinctions and honours, which included the Swiss Einstein Medal, awarded to him in 1970.

Gregarious and extrovert, his forthrightness and lack of false modesty endeared him to some, made enemies of others. His co-workers and many friends in different parts of the world enjoyed his unique kind of humour, were buoyed by

his passion for life, and stimulated by his enthusiasm for intellectual pursuits. Sentimental and deeply emotional, he was intensely loyal to individuals whose friendship he valued, expecting the same degree of allegiance in return. But perhaps the most enduring impression will be his automatic reflex to side with, and concretely support, human beings — whether fellow students in Germany, penniless intellectual exiles in Palestine, or anyone else who crossed his path — who were treated unjustly or were in need of help. He is survived by his wife, a daughter, and three sons, to whom we extend our profound sympathy in their great personal loss.

Alwyn van der Merwe

David Scott Gilbert

DAVID SCOTT GILBERT was born in Ithaca (N.Y.) on 6 November 1940. He majored in mathematics at Harvard University (1959-63) then, perhaps because of a biological tradition in his family, joined D.H. Fender at the California Institute of Technology, where he gained his doctorate on visual acuity and eye movements. His postdoctoral work was with the late Trevor Shaw at Queen Mary College London, where he later became a lecturer in zoology before joining, in 1973, the Medical Research Council Cell Biophysics Unit at King's College London. He died suddenly of viral pneumonitis on 11 December 1979.

While working with Shaw on sodium transport across the membranes of giant axons, Gilbert became interested in the structural properties of axoplasm and thus concerned with problems of the determination and maintenance of the shape of nerve cells. His first paper on axoplasmic structure in 1972, (*Nature, New Biology* **237**, 195-198) was remarkably stimulating and procedurally elegant. Using polarised light microscopy he showed that the giant axon of *Myxicola* (a marine fan worm) can be described in terms of three levels of helical organisation. The axoplasmic fibrous proteins are arrayed in parallel 'ripple' helices, which are twisted into a larger 'segmental' helix. They form a cylindrical gel that can coil, when the worm shortens during contraction, to form a yet larger 'gel' helix. Gilbert recognised that this axon consisted of essentially one structural component, the neurofilament, and therefore it provided an unparalleled opportunity for experimental study. The demonstration of the helical organisation led to a model in which the filamentous protein forms into twisted strands like a rope and, although the protein content of the axoplasm of *Myxicola* is no more than about 4%, the yarn gives it significant

mechanical strength and stability; and it can shear to form branches.

Many earlier observations of axons had been interpreted as demonstrating them to be a viscous soup, rather than this kind of stiff gel, and had led to the supposition that the mechanical properties were due to the membrane and associated connective tissue. Gilbert and his colleagues found the neurofilament gel to be solubilised by a very rapid enzymic autolysis triggered upon the entry of calcium ions into the axoplasm; the technique of very quickly extricating the axoplasm in air had avoided exposure to the calcium ions of physiological saline or sea water. This method enabled Gilbert (1975) unequivocally to determine many of its bulk chemical and physical properties (*J. Physiol. Lond.* **253**, 257-319). Thus the preparation provided an important standard of reference for working on the biochemistry and structure of filaments (10nm filaments, *Nature* **272**, 557-8) and formed a firm basis from which a long-needed attack on axonal structure at the molecular level could begin.

Gilbert was leading the developments on a wide experimental front at the time of his death. Fortunately much of his work with that of colleagues is drafted for publication. Using mammalian as well as cephalopod and annelid nerve cells they have uncovered a wide variety of different neurofilament proteins. To examine their homologies they have developed a new high resolution system of gel electrophoresis and are beginning to show by fingerprinting techniques that the variety of neurofilament chains in several species, including some mammals, conceals an underlying simplicity. With a view to uncovering the biochemical as well as the structural features of neurofilaments Gilbert and his colleagues have assayed their modification by the action of axonal proteases and of endogenous kinases and phosphatases. At the same time data from X-ray and solution studies are leading to the development of a detailed molecular model of the neurofilament.

Gilbert had a keen critical intelligence and an uncommon ability to choose significant and clear-cut biological problems. He mastered a wide variety of techniques and brought to them exceptional manipulative skill. In our laboratory his broad interests and strong grasp of physical principles made him much in demand for discussion and advice. He was infallibly helpful and generous. We, his colleagues, are impoverished by his death, which will grievously set back the development of this kind of neurobiology in Britain. David had an essential humility and natural friendliness that enabled him to enjoy an easy relationship with a wide variety of people. We mourn him as much because we have lost a friend, as because a young scientist of distinction has vanished from the international scene.

B.B. Boycott

27 March 1980

Foreign students: time for the government to do its sums

To judge by the pattern of previous years more than 90% of all applications for university places for the next UK academic year have now been made, and it looks, at first sight, as if overseas applicants have not been substantially deterred by the government's ill-considered decision to make this year's fees two to five times higher than last year's. The acid test, however, both for universities and other institutes of further education will come after the summer when they discover the rate of conversion of applicants into real students bearing the new fees. And since the omens do not look good, the universities are gloomily pondering the likely consequences.

There was, and still is, little evidence that much thought went into the possible consequences of last November's decision to raise the fees. Rather, the decision was part of the government's sweeping monetarist policies aimed at reducing public expenditure. The facts were that in 1978 87,000 overseas students attended UK institutions of further education (35,000 at universities) and that they were being subsidised by the British taxpayer to the sum of £100 million per annum.

In order to save the taxpayer that sum it was decided to charge overseas students the full cost of their studies. That meant £3,000 per annum for undergraduate science courses and £5,000 per annum for those undertaking medical, dental or veterinary studies. The fees for postgraduates were not stipulated, but many universities have decided to use the same rates.

The universities have been warned to expect their grants to be cut in the next academic year by an amount commensurate with their past intake of foreign students. It will be up to the universities to recoup their lost income from the students. The question is: what happens if much of the income does not materialize because the universities find themselves priced out of the market? — a distinct likelihood according to recent polls of extant foreign students in the UK that have been taken both by universities and student bodies.

There are certain prestige institutions whose very existence would be immediately threatened were their intake of overseas students to drop by the 40% to 90% that some of the polls suggest. The London School of Hygiene and Tropical Medicine with 75% foreign students and the Royal Postgraduate School of Medicine with 47% foreign students are particularly vulnerable. The Imperial College of Science and Technology and the University of Manchester Institute of Science and Technology would also have serious problems.

Even a considerably smaller shortfall in foreign students than the polls suggest will have serious consequences. In the first place it will inevitably exacerbate the academic job shortage, and make yet gloomier the prospect of easing the situation, given the demographic decline in home-grown students that begins in a few years' time.

Next, a shortage of foreign postgraduate students seriously threatens the quantity of research carried out in the UK, since many a busy academic runs his or her research project with one or

more hard-working foreign graduates. And they are irreplaceable because there is neither the financial support nor the moral justification — given current career prospects — to replace foreign graduate students with more from within these shores.

Another worrying prospect about the increase in fees is that it will not just deprive foreign students of an education in the UK, but that it will altogether hinder their chances of overseas studies. In particular, the many students from developing countries that were once British colonies who have taken advantage of the subsidised rates on offer in the UK may neither be able to afford the new fees nor to find the places or the cash to study elsewhere overseas. That would be not just a blow to the individuals and their countries of origin, but would also decrease UK influence in worldwide scientific and educational matters.

For the present, the government is adopting a policy of wait-and-see, possibly because it is not particularly concerned about the outcome either way. Thus, if it turns out that the new fees can be afforded by the majority of students, no harm will have been done. Whereas any substantial drop in student numbers can be used to justify a retrenchment of the funding of further education, a welcome opportunity for a government intent on cutting public expenditure. Presumably the government does anticipate a drop in overseas student numbers, because it has repeatedly emphasized that their number has almost trebled in the past decade, and that it currently exceeds by 15,000 the nominal quota set by the previous government. It has also argued that many of the current overseas students come from wealthy oil-producing countries well able to afford the new fees — an argument that ignores the plight of those who cannot and the fact that universities estimate that no more than ten per cent of their foreign students come from the wealthier nations.

The only concession so far made by the architects of the new policy is to provide the Committee of Vice Chancellors and Principals with enough money to provide bursaries covering the increase in fees to the 400 most able postgraduate students. Even that small but welcome concession is likely to be more than wiped out by the effects of last summer's 15% cut in the budget of the Overseas Development Administration which, in 1978, had supported in part or in whole more than 9,000 students from developing countries pursuing academic qualifications in the UK.

In the circumstances, what is urgently needed is some real idea of government thinking on the consequences of its policy. At present, even the very genuine concerns of such threatened centres as the Royal Postgraduate Medical School have been met with a stony wait-and-see response from the government, who could surely provide some concrete reassurance about their survival in the event of a serious loss of foreign students. In any case, faced with the contradictory evidence of the number of applications and the polls of students already here, it is surely time for the government to devise and carry out some real investigation of the rate at which foreign students will appear next academic year. □

United States

Research data: private property or public good?

Do scientists have a right to protect preliminary research findings from outside scrutiny? **David Dickson** reports on a growing controversy

LAST year, when a New York public interest group needed help in analysing the results of a national survey of infant-feeding practices, it turned to the Center for Disease Control (CDC) in Atlanta for assistance in putting the data through a computer.

CDC agreed to cooperate, and the data were duly stored. But being a public agency, its records are open to public scrutiny under the Freedom of Information Act. And CDC subsequently received a request from Abbott Laboratories and Mead-Johnson (a subsidiary of Bristol Myers), for access to the survey data. Ross Laboratories (a subsidiary of Abbott) and Mead-Johnson have been criticised over the nutritional value of their baby foods.

In January an administrative law judge upheld CDC's view that it lacks the legal power to deny the companies access to the data. And the centre is now being taken to court by the public interest group, the Interfaith Center on Corporate Responsibility (ICCR) to protect its data until it has been able to analyse the results and publish its own conclusions.

Two factors are complicating the increasingly sensitive problem of whether a research worker has any moral or legal rights over preliminary research data. The first is the growing economic importance of such data, for example where animal studies or clinical trials determine whether a new drug can be licensed. The second is the public's demand for information on substances likely to affect lives.

In the case of privately funded research, companies retain the right to withhold all test data supporting claims of efficacy or safety, on the grounds that these are trade secrets whose publication might have an economic impact on the product.

Moreover the Supreme Court, in a case brought by a group of physicians demanding access to data criticising the efficacy of an anti-diabetes drug, ruled last month that the raw data generated by a private laboratory under contract to the National Institutes of Health do not constitute an 'agency record' within the meaning of the FoI Act.

The main problem concerns the type of access permitted to federally-conducted research: in the case of clinical trials, for example, it can be argued that the disclosure of partial results before the trial is completed may jeopardise the final outcome.

Spurred by such concerns, NIH is seeking exemption for such data from the

Freedom of Information Act. Initially it had proposed that both epidemiological studies and clinical trials should be exempt. But following various objections, it has now developed a more restricted proposal, namely that it will be possible to withhold from public disclosure 'trend data' until it has been completed and verified (individual medical records are already protected by law).

To counter the argument that, without access to information on the conduct of the trial, the public has no check on whether it is being carried out responsibly, NIH is also proposing that exemptions will only be allowed if explicit measures have been taken to assure patients' safety.

Not all critics are satisfied. Dr Sidney Wolfe of the Health Research Group for example, which has consistently attacked the secrecy surrounding drug tests, argues that the protection of patients and the integrity of the tests can only be guaranteed through the fullest possible disclosure.

"Research workers can have an incredible bias, which may not be in the best interests of participants or intended participants in a particular trial" he says. "In general the more people who have access to a given body of data, the better".

Others argue differently, not only supporting the pragmatic claim that up-setting a large-scale blind trial may be very expensive, but also suggesting that a research worker has a moral right to present the first interpretation of data he or she has collected.

"This has been an underlying postulate of scientific conduct which is generally accepted" says Professor John Edsall of Harvard University, chairman of the

American Association for the Advancement of Science's Committee on Scientific Freedom and Responsibility.

Some also fear that, if a scientist's research notes are not protected from public scrutiny, he or she may be tempted to destroy them once the data has appeared in its final form.

The NIH's proposal that its clinical trials may, in given circumstances, be exempt from the full provisions of the FoI Act have been closely scrutinised by the NIH's Ethics Advisory Board. And following lengthy discussion of the revised proposal at a meeting of the board two weeks ago, it seems that the board will be prepared to support the proposal when it meets again in April.

It has been less convinced by arguments that information provided by private hospitals to CDC — for example on problems of infection in hospital wards — be kept secret for fear that publicity could generate undue public concern.

CDC had argued that, without some guarantee of anonymity, hospitals may be reluctant to come to them for assistance. But board members expressed the opinion that, although exemptions might be appropriate in some circumstances, this type of information was likely to have already reached the public domain by other routes.

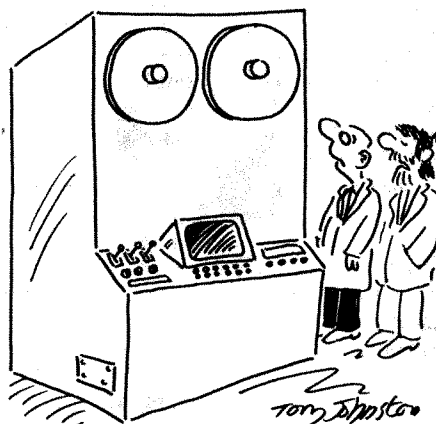
And then there is the case of the infant feeding survey results, private data whose presence in CDC's computers appears to have made it public.

Here the view that all information should be as open as possible conflicts directly with the claim that, particularly in such a sensitive area, those who have collected the data should be allowed to offer their interpretation before others try to refute it.

Indeed, in this case the two baby-food manufacturers concerned have already written to family practitioners suggesting weaknesses in the study, which was carried out to discover the extent to which lower-income families use processed baby foods, and the nutritional implications.

ICCR claims that, since it collected the data — and has agreed eventually to make it available to anyone interested — it has a right to prevent its prior use by others. And it quotes in support the practice of the World Health Organisation, namely that research data received for analysis and processing "will not be released in incomplete form to any third party without permission from the research scientist who has collected the data".

It is now up to a court of law to decide what force the traditions of the research community hold against current legal definitions of the rights of access to the results of research. □



"What makes you think the computer will be biased against processed baby foods?"

US universities are keeping pressure on federal agencies to reduce the burden of government regulation on the administration of research grants. The universities claim that over-zealous regulation has created a climate of distrust between themselves and the government.

Two reports emphasising the need for more sympathetic regulation have recently appeared in Washington, one arguing for a general relaxation of the rules governing how universities should account for the use of research funds. The other seeking institutional changes in the way that such rules are administered.

The first report, produced by the National Commission on Research, a body established in 1978 by a group of higher education institutions, says that universities should be given greater responsibility to regulate themselves. For example, rather than research workers being required to complete detailed reports of the way in which they spend their time, it suggests that there should be merely "explicit certification by individual investigators that direct salary charges to their research agreements are reasonable and fair, coupled with the federal program officer's review of the reasonableness of these expenditures for the work undertaken".

The commission, whose report appears in the 14 March issue of *Science*, also recommends that government agencies and universities should construct an option, analogous to the "standard deduction" in income tax calculations, to charge activity treated as indirect costs under sponsored agreements. The fixed percentage would be

Grants regulation: change urged

negotiated, and could either be uniform, or vary from one institution to the other.

More specific recommendations have come from the Sloan Commission on Government and Higher Education, a two-year enquiry financed by the Alfred P Sloan Foundation of New York. In general, says the Sloan Commission, support systems for university research are "fundamentally sound". But steadier funding and long-term real growth are needed: and like the NCR, the Sloan Commission criticises the excess of government regulation.

The Sloan report, which was published in Washington last week, recommends the development of a "corps of federal auditors sophisticated about scientific research and how research universities operate". It suggests that the "natural location" for a new audit agency would be in the National Science Foundation.

At present, most universities are audited by the Department of Health, Education and Welfare, according to a federal rule that auditing responsibility is assumed by the government agency which provides the largest financial support.

In recent months DHEW auditors have become increasingly critical of the way in which universities account for the use of research funds, pinpointing areas of

misuse and inadequate auditing. According to the Sloan Commission, however, DHEW's representatives "often have little experience in dealing with research institutions, receive little training, and typically stay in the assignment only briefly".

The commission suggests that the NSF is the most competent body to conduct financial oversight since it is "the only federal agency devoted entirely to research". (Although others have argued that its present close relationship to universities might preclude any credibility as an objective auditing agency.)

In other recommendations designed to strengthen federal support of university research, the commission recommends that every research grant and contract carry with it an additional 7% of the project's direct costs. These would be used in direct support of research, but on projects selected by the university and its faculty rather than the funding agency.

And in order to help alleviate the growing problem of faculty recruitment, the Sloan Commission recommends that about \$100 million a year be spent on two new kinds of fellowship to provide short-term support for PhDs. One would be a scheme of 1000 competitive national post-doctoral fellowships awarded each year, and carrying two years' support, and renewable for a further two years. The second would be a scheme of 300 national research fellowships, each carrying five years of support for research on university campuses, in federally-funded research centres and intramural federal laboratories. □

Antibiotics in animal feeds: health study 'may be impossible'

In a report that gives little direct guidance to policy-makers, a panel of the National Academy of Sciences has concluded that there is insufficient evidence to determine whether the use of sub-therapeutic doses of antibiotics in animal feeds is a significant threat to human health.

The report also states that the research necessary to establish and measure a definite risk "has not been conducted and, indeed, may not be possible."

The Academy's report seems likely to spark another round of a controversy that has simmered ever since the publication of the Swann report in the UK in 1968. This pointed to the dangers of antibiotic resistance being passed from animals to humans. Antibiotics in animal feedstuffs were subsequently banned in the UK, and later in other European countries.

Attempts to impose a similar ban in the US have been strongly opposed by the animal feedstuff and livestock industries. When the FDA announced in 1977 that it was considering such a ban, the proposal was withdrawn after a public hearing at which several companies expressed the view that there was insufficient evidence to demonstrate that such a ban was necessary

— and that if imposed it would considerably increase the price of meat.

The Academy's report, produced by a panel under the chairmanship of Dr Reuel Stallones of the University of Texas School of Public Health, provides little clue to the FDA about how it should proceed. But it does highlight areas in which more data are needed.

For example, the report says that those in close contact with animals receiving antibiotics "are more likely to harbour antimicrobial resistant *E. coli* than persons who are not exposed. However, studies do not usually indicate the type, duration and dose levels of the antimicrobials received by the animals: sub-therapeutic use was not distinguishable from therapeutic use".

Furthermore, the panel states, there are no data from which to assess the relationship between the consumption of meat from animals that received sub-therapeutic amounts of antibiotics, and the general prevalence of antimicrobial-resistant *E. coli* in human populations.

Nor, it suggests, can much help be gathered from studying the situation in Europe. "Data gathered from the United Kingdom, the Federal Republic of

Germany and the Netherlands do not indicate clearly whether restrictive regulations have actually reduced or averted the postulated hazards to human health", the report says.

And it adds that restrictions on the use of antibiotics in the United Kingdom "may well have altered the patterns of their use without significant alteration to the total amounts used or their consequences".

The committee does suggest four possible studies on individual aspects of the transmission chain — on the effects in animals, on the passage of antibiotic resistance to meat-eaters, on the effects of occupational exposures to animals, and on the human morbidity and mortality consequences of antibiotic resistance — which, it says, "would provide a useful scientific background for policy-makers".

But the panel warns that at best "the remaining gaps in our knowledge will still have to be bridged by conjecture and speculation". This is little consolation to the FDA or to Congress, which has already provided a further \$1.5 million in the Agency's current budget for a full-scale comprehensive survey.

David Dickson

Soviet Union

Incident at Military Village No. 19

LAST week the Soviet Union for the first time admitted that an outbreak of some killer infection occurred last year in Sverdlovsk. Replying to a question from the US State Department, the Soviet authorities confirmed that an outbreak of anthrax did occur, but maintained that the disease was caused by poor food handling, not by bacteriological warfare agents as rumoured in the West. Since the Soviet Union has signed the international convention banning the use of such agents, this denial comes as no surprise. What is perhaps most significant is that the Soviet authorities have admitted that some bacteriological incident did take place after almost a year of "no comment" or "nothing happened".

Sverdlovsk, on the eastern side of the Urals, is a "closed city" to which foreign visitors are not admitted. Local information reaching the West is necessarily at least second-hand, and it would be unwise to give undue credence to every detail. The details on which the US State Department based its enquiry seem to be drawn from a single Russian underground source — albeit one which claims to emanate from Sverdlovsk.

However, even one such document is worth taking seriously — if not as evidence of what occurred, at least as a pointer to local fears and responses.

According to this document, in April 1979 there was an escape of a bacterial strain called V-21 in "Military Village No. 19" on the south-western outskirts of the city. The bacteria were carried by a north wind to the village of Kashino, with only a negligible amount reaching Sverdlovsk proper.

Casualties were mainly among the military and civilian residents of "Military Village No. 19", inhabitants of Kashino, and workers at the local ceramics plant. Death typically occurred some 1-3 hours after hospitalisation, following a fever of over 42°C. Special army nurses wearing protective clothing were brought in. Bodies of those who died were not returned to the relatives for funeral ceremonies. The total death toll was unofficially estimated at over 1,000.

Official local reaction, says the report, took the form of placebo messages, which gradually escalated from "nothing is happening" at the beginning of the outbreak, to "nothing happened, and anyway, don't panic, it is all localised and under control" towards the end. Finally, the streets of Kashino village were paved with asphalt, apparently to neutralise remaining infection.

Equally circumstantial are rumours emanating from Slovakia of a similar incident involving a Soviet military base.

Here, however, there were no fatalities, only a widespread outbreak of what appears to be a new form of infective hepatitis. According to local sources, an area of central Slovakia some 70 km by 30 km is regularly used by Soviet paratroopers as a training ground. Some time in late August or early September it is alleged that one such training exercise led to the accidental discharge of bacteriological weapons. The military casualties were said to be too numerous for the existing army facilities, and the

overflow was taken to civilian hospitals in Poprad and Spisska Nova Ves.

Inevitably some of the virus was borne by the wind outside the training ground, and "several thousand" of the civilian population also succumbed. The Slovak sources stress that this does not appear to have been intended as a killer weapon — rather one to debilitate and incapacitate the local population and thus minimise resistance before the arrival of an unwished for "peace-keeping" force.

Vera Rich

Nuclear energy



Loch Doon, Scotland: UKAEA refused test drilling permission

Power dissenters through Europe

AN overwhelming majority of Swedes voted against any further extension of nuclear power in a special advisory referendum last Sunday. By a combined vote of 76% to 19% with 5% undecided, the electorate registered its approval to phase out Sweden's present nuclear programme within 25 years. Thirty nine per cent of the voters supported the Centre Party-Communist proposal to dismantle the programme within 10 years, 37% supported the Social Democrat-Liberal proposal to use present and planned capacity for 25 years as an emergency measure only, and 19% supported a Conservative option that would leave the door open for the development of uranium mining and the fast breeder.

● Swiss voters in the village of Hagedorf in the north west canton of Solothurn may have blocked effectively further nuclear development in Switzerland last week by refusing to give permission for test drilling for nuclear waste disposal. The 2,500 villagers rejected proposals by the State-backed company Nagra to drill for waste disposal sites by a 3-1 margin, despite

having voted in favour of the government's energy programme last year.

● French residents in the Brittany towns in cap Sizun claimed a victory last week when the provincial prefect at Quimper freed seven anti-nuclear demonstrators arrested during running battles with the police at protests on 29 February. A crowd of several thousand residents shouted "we have won" at the 1,500 police stationed around the Quimper courthouse. M. Jean-Marie Kerloc'h, mayor of Plogoff, called the decision "comforting". After the court decision and the pre-trial mass demonstration of 40,000 people, it is now widely believed that the central government will be forced to back down from its attempt to impose a complex of four nuclear reactors on the strongly nationalistic Breton people.

● In Finland 5,000 demonstrators, including a large number from the countryside, marched through Helsinki last week to show their solidarity with Swedish anti-nuclear protests and their opposition to the construction of a Soviet

designed 1000MW plant. The demonstration was the largest in Finland since the anti-Vietnam war demonstrations of the late 1970s. Finnish environmentalists are demanding that the contract between the state-owned Imatra Voima company and the Soviet export organisations, Atomenergoexport and all other future nuclear decisions be subjected to full parliamentary debate.

● Dutch police arrested 29 anti-nuclear demonstrators who chained themselves to the gates of the Borssele nuclear plant in Zeeland last week. The protest prevented two changes of shift at the nuclear facility. A total of 150 demonstrators, members of the Dutch anti-nuclear group "Break the Netherlands Atomic Chain" padlocked all seven gates at the plant, preventing entrance for 20 hours until their arrest and dispersal by police.

● In Britain, a second Scottish local authority has refused planning permission for test site waste disposal drilling. The Atomic Energy Authority has asked for another public inquiry to be held to try to obtain permission to drill 32 boreholes near Loch Doon, south west Scotland after its proposal was rejected by the Kyle and Carrick county council. The Conservative-controlled council feared that if granted, the proposal would lead to a detailed application for a demonstration disposal plant.

● According to figures released by the Secretary of State for the Environment in parliament recently, the UK civil and military nuclear programmes produce 100m³ of high level liquid wastes, 500m³ high level solid wastes, 450m³ plutonium contaminated waste and 250m³ miscellaneous waste each year. Some medium and low activity wastes including plutonium-contaminated material and reactor decommissioning wastes are dumped at sea.

The total waste stored at civil nuclear power stations at the end of 1979 was 20,000m³. Of this, the total accumulation of high level liquid waste was 1,000m³ at Windscale from the reprocessing of power reactor fuel and 700m³ at Dounreay from the reprocessing of fuel from experimental reactors. In addition, approximately 9,000m³ of high level solid waste and 3,500m³ of plutonium-contaminated waste are in store. The total accumulation at AEA and British Nuclear Fuel sites of medium level liquid wastes, concentrates, sludges and resins, wastes from decommissioning reactors and other plant and other medium level wastes is about 11,000m³.

The National Radiation Protection Board has spent £70,000 since 1977 to assess the radiological consequences of disposing of radioactive waste in geological formations.

Joe Schwartz

EEC

Melt-down experiment go-ahead

FRANCE has removed its objections to an experiment simulating the melt-down of a nuclear reactor, and so removed the last obstacle to the European Community's £530 million, 1979-83 programme for fusion research and support of the Joint Research Centre at Ispra, North Italy.

Italy, as part of its diplomatic activity over European research (3 January, page 3) had refused to endorse the budget of the EEC's fusion programme until France accepted the meltdown experiment, which is now to take place between 1983 and 1986 in the reactor Epsilon at Ispra.

France was planning its own similar experiment at Cadarache, but, says Mr Tom Doyle, head of Epsilon division, "Cadarache has only half the dimensions of ours" and the scale of the experiment substantially affects the results.

The US exerted pressure for the Epsilon experiment — called 'Super-SARA' — to be undertaken as a next step from similar, smaller tests which have been performed on the Power Burst Facility at Idaho. According to a 1979 Nuclear Regulatory Commission report Super-SARA should provide more information on melt-down than any other planned experiment.

"Epsilon is a heavy water reactor" Mr Doyle

told *Nature*; "the active core is 1.5 metres high, compared to a typical research reactor's 0.8 metres." Moreover there are enough channels to make simultaneous tests on 36 fuel rods (compared to 16 at Idaho).

Super-SARA will consist of 20 experiments to simulate loss of coolant in a light water reactor either rapidly, over 10-20 minutes, or more slowly (the 'small break' case as at Three Mile Island). Fuel rods will be taken to the rupture of cladding, but it is intended to leave the rest of the reactor unaffected. This is "a bit of a difficult problem" says Doyle, but not insurmountable.

The experiments are necessary, he says, because calculations are very difficult: "you have a two-phase problem under transient conditions".

The successful flurry of diplomatic activity between Italy and France over Super-SARA began last December, when the EEC Council of Energy Ministers was faced with a French veto. The French High Commissioner for Atomic Energy in the case was Jean Teillac who, as President of the Council of the European sub-nuclear physics organisation, CERN, was resisting Italian moves to 'clarify' CERN's future.

Robert Walgate

UK attacks mercury directive

THE latest draft environmental directive issued by the European Commission in Brussels concerns mercury emitted into rivers by the chlor-alkali industry — and it has come in for the now familiar sharp attack from the UK House of Lords sub-committee on the environment.

In a report published last week, the sub-committee charges that the EEC directive should not confine itself to one industry (there are other sources of mercury), should deal separately with solid and soluble mercury, and should not specify limits without indicating whether an absolute maximum or some kind of average is intended. Moreover, some of the limits are unduly stringent, says the report.

However, Britain emits more mercury into its rivers than any other EEC country, when the figures are related to chlorine production: 16.9 g per tonne of chlorine compared, say, to 3g per tonne in France.

But the basic difference between the Lords and Brussels is over the difference between "environmental quality objectives" (EQOs) as usually adopted for water in Britain, and the "emission standards" favoured by other EEC members. Although Britain has convinced the Commission to establish both for each

new pollutant considered (mercury is the second, after aldrin, dieldrin, and endrin, and cadmium will be next) the philosophies of the two are too different for them to sit easily together.

EQOs are easiest to establish where the control of river catchment areas is unified, as it is through the water authorities in the UK, and water use, defining necessary quality, a national matter. But in continental Europe rivers such as the Rhine are far from such unification.

"The apprehensions of member states subject to cross-frontier pollution" says the report "rest partly on the fact that an upstream member choosing the EQO alternative route could adopt a water use requiring less stringent standards than a neighbouring downstream member state obliged to use the water for purposes . . . which would demand higher standards".

Britain and the rest of the community are thus likely to have continuing difficulties over environmental standards; and these are accentuated by the greater scientific rigour required when setting EQOs as opposed to emission standards. "It is clear" says the House of Lords report "that the Commission has too few staff available to work on environmental proposals . . . For example, there is no expert on toxicology." The House of Lords will debate the report on 17 April 1980.

Robert Walgate

'Water pollution: mercury' House of Lords Session 1979-80 38th report of the Select Committee on the European Communities. HMSO.

NEWS IN BRIEF

US-Yugoslav interferon agreement

THE National Patent Development Corporation, a private company which is planning to build a pilot plant in New Brunswick, New Jersey, to produce interferon, last week announced that it had signed an agreement with the Yugoslav Academy of Science's Institute of Immunology for regular supplies of the protein.

Under the agreement, National Patent will receive 4.5 billion international reference units of the human leukocyte within the next six months. And within that period, an accord will be reached under which National Patent will be supplied with 10 billion units a month.

Meanwhile the *Boston Globe* reported last week that research workers at the Massachusetts Institute of Technology expect to announce shortly that they have developed a method for producing interferon at only five per cent of current production costs.

The process is said to be based on research carried out in MIT's Department of Nutrition and Food Science, which has shown that human cells can be grown on small beads of starch, and that such a process can be used in particular for cells producing interferon.

Although the efficacy of the technique has yet to be demonstrated, the process has already been licensed exclusively to Flow Laboratories of Virginia. The company is currently negotiating with the National Cancer Institute to supply 50 billion units of interferon using the MIT process, which is claimed to reduce the cost of a million units from \$50 to \$2.50.

The CIA's toxic agents

BIAGEN — the European-based genetic engineering company largely owned by International Nickel and Schering Plough, which recently announced the successful synthesis of interferon — may have a less illustrious predecessor. According to information made public recently by the Church of Scientology, the Central Intelligence Agency developed a machine called Biogen in the late 1950s to manufacture toxic organisms for use in covert biological warfare.

The scientologists, who have been conducting an active campaign to discredit the activities of the CIA, say that the machine was used for 13 years, and may have produced hundreds of pounds of various biological agents and microorganisms, in particular those capable of causing undulant fever and tularemia.

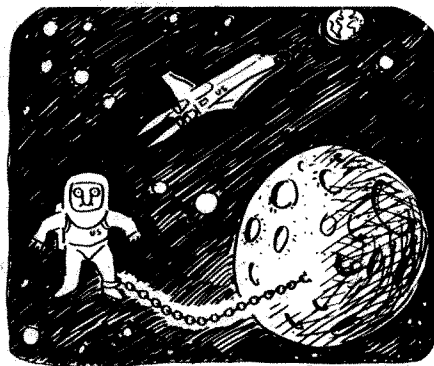
The scientologists also claim that the machine was kept in operation until 1972, three years after President Nixon had publicly renounced the use of biological weapons in warfare.

Frosch approach to space delinquency

COMMANDERS of the National Aeronautics and Space Administration's space shuttle, now due for its first flight early next year, will have the authority "to use any reasonable and necessary means, including physical force if necessary," to maintain order on board his craft, NASA administrator Dr Robert Frosch agreed last week.

Dr Frosch approved a new rule that would give the commander of the space shuttle powers to arrest any member of the space crew — including space scientists on board — and charge him or her with a crime punishable by a \$5,000 fine, a year in prison or both.

Agency officials argue that such rules were not necessary on earlier space flights involving fewer crew members and more constrained surroundings. But the space shuttle will carry seven individuals, four of whom will be civilian scientists. And according to NASA General Counsel S Neil Hosenball, the new situation demands both a formal chain of command and regulations "concerning possible criminal behaviour in space".



As a precedent, Mr Hosenball quoted the case of a technician who had been killed on an ice island in the Arctic after going berserk and threatening other members of a research team tracing the movement of ice-flows. A manslaughter charge was reversed by the Alaska Court of Appeals, which ruled that it lacked jurisdiction over a crime committed on an island that spent virtually all its time floating in the Arctic Ocean.

Oil drills escape US export control

AFTER several weeks of intense debate, the Carter administration announced last week that it is planning to tighten controls on American exports of high technology products to the Soviet Union. According to a statement issued by the US Commerce Department, the new controls will cover areas such as computers and software, manufacturing technology, and "materials critical to the manufacture of high-

technology defense goods".

The new policy follows an extensive review of the government's attitude towards high technology exports, initiated in response to the Soviet Union's intervention in Afghanistan in December. In the area of computers, for example, administration officials stated that standards will be tightened to conform to those of 1976, when restrictions of computer exports to the USSR began to be released.

One exception to the ban will be on the export of oil drilling equipment to the Soviet Union. The administration had been advised that to cut off such exports, in a field where USSR technology is said to be many years behind that of the US, would only encourage the Soviet Union to seek oil supplies in other countries, and in particular to look towards Iran.

Radiation protection on a budget

IF one man-Sievert of radiation were distributed uniformly over the British population, resultant cancers would be thinly distributed and there would be little public reaction. But if the radiation were concentrated in one town, there would be no more cancers (if response is linear to dose) but there would be an outcry because of their concentration.

Considerations such as these have led the UK National Radiation Protection Board to make estimates of the amounts that it would be cost-effective to spend to protect Britain from unit amounts of radiation dose — if it were uniformly or non-uniformly distributed. One man-Sievert uniformly spread in Britain is worth £100,000 of protection, estimates the report (though it could be a factor of five either way). But concentrated in a town of 10,000 people it would deserve protection of £40 million.

NRPB admits its figures 'have no absolute significance', but are intended to be the basis for consultation.

'The application of cost-benefit analysis to the radiological protection of the public'
NRPB, Harwell, Didcot, Oxon OX11 0RQ

HSE gets Coalite report

THREE reports discussing the effect of 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) on workers at the UK firm of Coalite and Chemical Products were handed over to the Health and Safety Executive (HSE) last week. Two weeks ago *Nature* reported Coalite's reluctance to make this information public (5 March, page 2). The report now in the HSE's possession discusses immunological, biochemical and chromosome studies of workers exposed to dioxin at Coalite between 1968-71. An HSE spokesperson is quoted as saying that the Executive is now "assessing and appraising" the reports.

FEATURES

Distorting the epidemiology of cancer: the need for a more balanced overview

The politics of cancer are becoming increasingly polarised with environmentalists taking one extreme view and industrialists the other. **Richard Peto** argues that a more dispassionate and disinterested approach is required for a realistic assessment both of the risks from environmental carcinogens and of the preventative regulations needed

THERE exist, both in the general environment and in certain occupations, chemical or physical agents which increase the likelihood of human cancer. Our political response to this would, in an ideal world where sufficient knowledge was available, depend on the direct and indirect costs of the various possible measures of control, and on how many cancers each such measure would prevent. In the real world, estimates of the direct financial costs of the control of particular agents can easily differ by one or two orders of magnitude, the indirect costs may be grossly exaggerated, or some major indirect costs may be completely overlooked. Worst of all, we have in general no remotely reliable estimates of the numbers of cancers which particular legislative controls would prevent. Consequently, there is wide scope for pressure groups to have considerable influence on public policy.

Historically, the most powerful pressure groups in US society have been the large financial interests, which have almost always put financial advantage before human health. Over the past few years the major tobacco companies have launched massive sales drives in the Third World, which if successful will kill millions of people. (About one in four regular cigarette smokers is killed prematurely by smoking.) In the US, where cigarettes cause well over 100,000 deaths every year from lung cancer or chronic obstructive lung disease, the tobacco industry refuses to accept in public that cigarettes cause either disease, let alone to collaborate in serious public information, and no epithet can suitably describe the collective efforts of their advertisers.

No other industry kills people on anything like the scale that the tobacco industry does, but where other industries have been found to cause cancer (or dust-induced lung disease) in their workers or in

the consumers of their products, their immediate response has usually been to delay acceptance of the findings, to minimise their relevance to current practice, and in general to delay or obstruct any hygienic measures which will cost money. Even when human danger has been unequivocally demonstrated, industrial consortia may actively lobby for controls so weak that (as with the new UK government regulations limiting inhaled asbestos to 1 fibre/ml from 1981) they leave no reasonable safety margin. Large amounts of money are available to mount press or TV publicity campaigns about the homely apple-pie virtues of asbestos, journals financed by the tobacco industry run populist articles which misrepresent research results to lay readers, and some US television journalists are explicitly told always to censor all reference to the dangers of smoking.

Even the scientific literature is not immune from distortion by financial interests. The decades-long argument that "threshold" dose levels of carcinogens must exist below which the general population is absolutely safe has not been entirely motivated by the scientific plausibility of the hypothesis. With increasing understanding of the derivation of tumours from single cells acted on by mutagenic carcinogens, industry is slowly abandoning "threshold" arguments in favour of arguments^{1,2} (where the biological fallacies^{3,4} are somewhat better concealed by the mathematics) that thousandfold reductions in dose can conveniently be "statistically guaranteed" to produce a million-fold or some other enormous reduction in risk.

Even if the scientists who propound such models are disinterested, industrial endorsement of them is not. Much excellent toxicology may be done by industry, and many industrial scientists and managers may be directly and honestly concerned with the prevention of hazards. But so many examples of financially-motivated bias exist that the motives and work of industrial scientists and

consultants are inevitably distrusted.

Over the past quarter century, the "environmentalist" camp in North American society has pressed for (and achieved) stricter control of particular carcinogens in the work-place, the general environment, and the diet. Usually, their efforts have been directly resisted by the relevant financial interests. Inevitably, in view of the scantiness of reliable quantitative knowledge about the cancer risks to man of nearly all carcinogens, the argument has polarised. In the politics of cancer, each side takes a very extreme position. Industry usually argues for the irrelevance to man of animal or *in vitro* cancer tests, or minimises the quantitative hazards and exaggerates the costs of control. The environmentalists usually exaggerate the likely hazards and are largely indifferent to the costs of control.

'The most powerful pressure groups in the US have been the large financial interests which have almost always put financial advantage before human health'

The vacuum of reliable scientific knowledge is such that each side can find scientists who will maintain in courts, in public hearings or in the scientific literature whatever is politically convenient, and it is important to recognise that scientists on both sides of this debate now have career interests at stake in it.

A particularly good example of the biased writings of politically active environmentalists is Samuel Epstein's *The Politics of Cancer* (Sierra Club Books, 1978, revised in Anchor Press, 1979). Epstein outlines, for university-educated but not necessarily science-educated readers, his view of our present state of knowledge about prevention of death from cancer. He concludes that the cure rates for the major cancers have not been improving much over recent years, but that we do know enough now about the causes of cancer for the testing and regulation of environmental contaminants to prevent the majority of US cancer deaths, and that the main obstacles to our doing so are political rather than scientific. He therefore reviews the scientific and political circumstances surrounding a

Richard Peto is a reader in cancer studies at the University of Oxford. This article developed from a review of Samuel Epstein's book, The Politics of Cancer

dozen or so disputed consumer products or occupational hazards, providing much fascinating political detail, and then describes at length the internal politics of the various agencies which research, regulate and litigate in Washington.

Epstein's book has been written to inflame political passions against environmental carcinogens, and parts of it are well worth reading. However, the political punch is often achieved at the expense of scientific accuracy and balance. Despite this, the book has already gained wide and apparently uncritical acceptance even among scientists. For that reason, it is worth considering some of the misrepresentations of scientific evidence which it contains, and some of the more general defects in the environmentalist perspective on cancer.

First, a few details. Epstein's book is very useful as a source of reference to original papers, but it is not in itself a reliable secondary reference because the material presented is so often distorted. Sometimes this distortion is due to the effects of Epstein's vigorous campaigning style on his scientific judgement and is, perhaps, forgivable; at other times it appears to be deliberate, which cannot so easily be forgiven.

For example, consider the (by now generally agreed) finding that incorporation of about 5% by weight of saccharin into rat diets gives a few rats bladder cancer, but has no generally accepted effects on any other type of cancer. By human standards, 5% represents a vast saccharin intake (and so, of course, the industrial "Calorie Control Council" have tried to shrug off these findings). To refute the common reaction that "anything given in large enough doses will cause cancer in animals", Epstein's chief argument is to report that 0.01% saccharin has also been shown to be carcinogenic. In support of this extraordinary claim, he presents in tabular form the control and 0.01% data for selected cancers from certain multi-group feeding experiments, leaving out the observations from those same experiments which would

have refuted it (see table). This appears to be a deliberate attempt to deceive the reader. It is not a casual slip in a 600-page book, as Epstein devotes twenty pages and two full-page tables to saccharin. His entire table 6.4, entitled "Tumours other than the bladder in rodents fed saccharin", appears to be so subject to artefacts of selection that it provides no evidence that saccharin does cause any rodent tumours other than in the rat bladder.

Saccharin is not an isolated example of bias; indeed, I found that in many places where he discussed data with which I was familiar inaccuracies were present, almost always in the direction of accentuating the need for battle with the devils of industry.

Turning to more important matters, Epstein asserts that any benefits from the "less dangerous" cigarettes which the tobacco industry has developed will be outweighed by people increasing their consumption to get more nicotine, and he is therefore in many places particularly scathing about (or even downright opposed to) research into changed cigarette composition. This is a distorted perspective on one of the more promising immediate means of preventing fatal cancers, a quarter of which in America are currently due to smoking.

Smokers of "less dangerous" cigarettes have already been found in various epidemiological studies to have disease rates which are materially lower than smokers of other cigarettes^{6,7}; at autopsy they have far fewer "pre-malignant" histological changes in their bronchi⁸ and, perhaps due to the changes in cigarette composition 10 or 20 years ago male lung cancer death rates in early middle age are now decreasing in North America, in Britain and in Finland.

After lung, the next commonest fatal cancers in the US are those of the breast and the large intestine, for which the most striking epidemiological finding to date is a 90% correlation between fat consumption in different countries and their breast or colon cancer rates (see figure; similar correlations exist for colon cancer¹⁰). Epstein suggests that dietary fat may

'Scientists on both sides of the environmentalist debate now have career interests at stake'

merely be acting as a vehicle for fat-soluble pesticides and industrial chemicals, which predictably emerge as his villains yet again. However, his suggestion is implausible: countries with high levels of contamination do not stand out from the general cancer/fat relationship, colorectal and breast cancer were common long before the widespread use of pesticides, and in experimental animals high-fat or low-fat diets can greatly enhance or reduce the risk of cancer¹¹. Something more interesting is waiting to be discovered in our diet than simple contamination by carcinogens.

The fat-associated and smoking-derived cancers collectively account for more than half of all cancer deaths, and people concerned with cancer politics should try to understand them considerably more accurately than Epstein appears to. One can list many other instances where Epstein's content or style are misleading or unbalanced. For example:

- The discussion of alcoholism and cancer is entirely erroneous because of failure to standardise properly for age.

- One of Epstein's chapters ends: "Eisenhower warned against the growing national threat of the military-industrial complex. The medical-industrial complex now appears to be as serious a threat".

- There is idealisation of the value of long-term animal tests with concomitant denigration of the value of the Ames test and other short-term tests. This is one of Epstein's most inexplicable errors of scientific judgement, unless he wants cancer tests to be difficult and expensive for industry.

- He is irritatingly puritanical, sneering at "the plastic age which symbolises how the value of our lifestyle has been degraded".

- He seeks by stylistic tricks to attribute to oral contraceptives some of the established hazards of hormone replacement therapy and of diethylstilboestrol.

- He claims that cancer costs the US economy over \$25 billion per year. (In fact, cancer must be prevented for humane, not for economic reasons; without cancer, there would be three or four million more retired North Americans to support, costing over \$25 billion per year).

And so on. He seems so certain about everything — how can anybody be justified in being so certain about so many details? Sometimes I know he's wrong, but more often (especially in the many places where inconclusive scientific results are presented as established fact) I know that nobody knows for sure. Lewis Thomas once wrote, in another context: "The sceptics in medicine have a hard time of it. It is much more difficult to be convincing about ignorance concerning disease mechanisms

Table: Data from OTA report⁵ on the tumour sites selected for presentation in Epstein's book to substantiate the claim that 0.01% saccharin is carcinogenic. Groups selectively omitted in Epstein's book are marked with an asterisk. These omissions substantially alter the implications of the original data.

Saccharin dose (% in rat diet; these doses have no material effect on longevity)	Male or female lymphosarcomas (FDA, 1948)	Female breast (FDA, 1973)	Male breast (FDA, 1973)
0 (control)	0/20* (0%)	6/26 (23%)	6/29 (21%)
0.01	8/14 (57%)	14/30 (47%)	14/25 (56%)
0.1	5/16 (31%)*	13/34 (38%)*	9/27 (33%)*
0.5	2/15 (13%)*	—	—
1	1/18 (6%)*	12/30 (40%)*	8/27 (30%)*
5	10/17 (59%)	12/27 (44%)*	7/25 (28%)*

*Omitted in Epstein's book.

⁵In addition to these 20, since some other sweeteners were being tested concurrently, 34 other control animals were studied in the same experiment, and the 1977 OTA review of these data, considering all the control animals to be equivalent, cited 9/54 control lymphosarcomas. (0/10 saccharin controls of each sex were studied, not 0/20 as Epstein inadvertently indicated.)

than it is to make claims for full comprehension, especially when the comprehension leads, logically or not, to some sort of action. When it comes to serious illness, the public tends, understandably, to be more sceptical about the sceptics, more willing to believe the true believers."

Epstein (like many others) is unjustifiably definite about three major issues. He seems certain that even after the effects of cigarettes have been allowed for, Americans live in an era of rapidly increasing cancer rates. But trends in recorded cancer death rates (and, perhaps more so, recorded incidence rates) over the past quarter century are biased upwards (since the cure rates for the major cancers have not materially improved) by improvements in medical care and cancer counting. These improvements have

'The NCI-NIEHS report shows how a group of reasonable men can collectively generate an unreasonable report'

affected all sectors of US society, but most particularly old people and previously poor people, especially blacks. Epstein, along with most other US commentators, does not allow for these biases even to the limited extent of restricting his attention to trends in age-specific mortality rates among middle-aged whites, where they might be expected to be least prominent.

Among middle-aged whites during the past quarter century, cancer cure rates have not materially improved, but some US cancer mortality trends seem to be genuinely down (stomach, cervix) while some seem to be genuinely up (pancreas, melanoma, and certain lymphomas). I can discern no net pattern other than that due to the massive effects of smoking on lung cancer, although no extension to 1978 yet exists of the excellent 1950-67 trend analyses in NCI monograph 33. In Britain, the same is true¹² as long as we examine mortality in middle age. (Interestingly, although Epstein emphasises that bladder cancer death rates among whites in the industrial north-eastern US are high, this may not be chiefly due to any current industrial hazard, for they have been decreasing both relatively and absolutely for a quarter of a century¹³.)

Epstein also seems certain that the majority of human cancer is caused by chemical and physical agents in the environment and could be prevented by their testing and regulation. There is no sound scientific basis for this certainty. Since the cancers (lung, breast, large intestine) which are commonest in the US are rare in certain other countries, and *vice versa*, they probably really are preventable, but (see figure) not necessarily by regulation of any environmental pollutants

or food additives. Indeed, one of the most intriguing animal findings is the effect in many studies of gross aspects of diet on cancer; for example, mice randomised between 5g of food once daily and *ad lib* consumption averaging 6g/day of the same food, had respectively 8% and 64% spontaneous mammary tumours¹⁴, and the role of dietary fat¹¹ has already been discussed.

Thirdly, Epstein seems certain that at least a quarter of all cancer deaths are attributable to occupational exposure to carcinogens. There is as yet no good evidence for hazards of anything like this magnitude, and there is clear evidence that many of his particular claims are exaggerated. For example, he says that asbestos has led to approximately 50,000 deaths per year in the US from cancer and related disease (referencing a paper by Selikoff¹⁵ which actually neither makes nor implies any such claim). This is sheer nonsense; of 50,000 asbestos deaths, several thousand should be certified as being due to pleural mesothelioma, yet only several hundred per year are thus certified.

Likewise, Epstein cites with approval (indeed, he describes it as being of "epochal significance") Health, Education and Welfare Secretary Califano's absurd 1978 estimate, in a speech to labour union leaders, that as many as half of the 8-11 million workers who have been exposed to asbestos could develop serious diseases such as cancer or asbestosis. In his second edition, Epstein draws extensively on Califano's source, a curious but extremely influential document which has been circulating privately for the past year or more. This unpublished typescript, with no listed authors, was prepared by a working group of nine well-known scientists including Arthur Upton and David Rall, the National Cancer Institute and National Institute of Environmental Health Sciences directors,

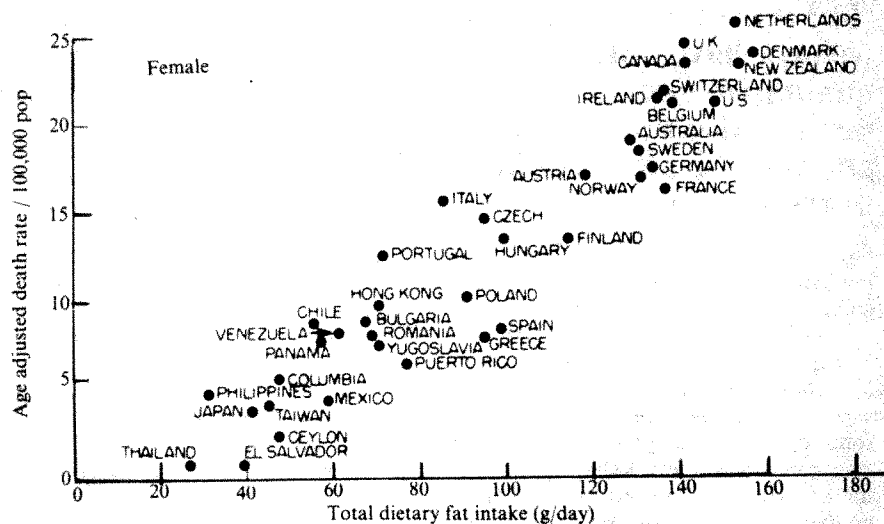
'Present day cancer rates and trends are probably not dominated by occupational carcinogens or environmental pollutants'

both of whom still seem more ready to defend than to repudiate it.

Entitled "Estimates of the fraction of cancer in the US related to occupational factors", it shows how a group of reasonable men can collectively generate an unreasonable report. The group multiplied the 12 million workers exposed in any way in 1972-4 to any of asbestos, As, Ni, Cr, benzene or petrochemicals by the very large risk factors (e.g. fivefold lung cancer excesses) typical of much more extensively exposed industrial populations, finally multiplying again by "4-5" to allow for the additional cancers that will be caused by these six agents among workers exposed to them at times other than 1972-4. (In casual disregard of dosimetry, this whole process is very like predicting 100,000 occupational lung cancer deaths per year among non-smokers due to their exposure to other people's cigarette smoke at work, "since 20% of heavy smokers get lung cancer").

These assumptions "predict" over 200,000 extra respiratory tract cancers per year in the near future due to the above six agents alone (and are the sole source of the currently widespread prediction of "20-38%" of cancers being due to occupation). But, in reality, fewer than 90,000 males/year get respiratory tract cancer in the US (and most of these are due to cigarettes and not to occupation at all), and moreover the US male rates in the first 25 years of working life are currently decreasing! A prediction of 100-200,000 extras due to the above six agents is clearly

Figure: data⁹ plotting for 39 countries age-versus total dietary fat (animal or standardised breast cancer death rate vegetable).



very unrealistic.

The unreality of the whole method is further highlighted by the fact that the assumptions "predict" $7,300 \times 4.5$ nickel-oxide induced occupational lung cancers/year. Since nickel refining causes a lot of nasal as well as lung cancer, we would presumably then also soon "expect" something like 13,000 nasal cancer death certificates per year among nickel workers. Nickel has already been widely used for decades, so many thousands of these should presumably already be evident. In reality, each year in the US population there are only 300-odd male and 200-odd female nasal cancer death certificates. The majority of these are not ex-nickel workers and no marked trends are evident. This demonstrates the unsoundness of the NCI-NIEHS methodology.

Unfortunately, such exaggerated estimates are so much what many people want to believe of modern society that they have now achieved a life of their own, and although they are utterly without foundation they are quoted widely, repeatedly and reputably both in the lay and scientific press. In Geneva the International Labour Organisation reportedly endorsed them, as did the Toxic Substances Strategy Committee in their recent draft report to the US president¹⁶, and they have been used in OSHA to emphasise the need for stricter laws. Most recently, the UK trade union the Association of Scientific, Technical and Managerial Staffs (ASTMS) released a policy document which appears to have been strongly influenced by Epstein's book, and which is notable for its prominent but erroneous assertion, derived solely from the foregoing unpublished US typescript, that 20-40% of current UK cancer deaths are caused by occupational carcinogens.

Epstein now claims that these exaggerated estimates seriously underestimate the impact of industrial carcinogens. There is obviously a valid political need to exaggerate the importance of the preventive measures we can already

death rates¹² should be more widely known.

My criticisms of Epstein's science, however, must be viewed in the light of the continued resistance of many industries to reasonable controls. One has only to read some of his descriptions of industrial behaviour to see where his passion comes from, and a suitably sceptical reader could derive much important information from the dozen or so detailed case-histories of particular carcinogens that make up the bulk of this book. But, although I cannot prove it, I suspect that environmentalists as a whole (including Epstein and those US and British trade unions which have already modelled their public statements closely on his book) would be better

'The politics of cancer is dominated on both sides by exaggeration'

respected and could therefore press more effectively for controls in particular instances if their overview of cancer was more balanced in the following ways:

- if they accepted that present-day cancer rates and trends are probably not dominated by occupational carcinogens or environmental pollutants (especially since they could still warn that future rates might perhaps be so dominated unless present-day exposures are regulated²⁰);
- if their chief concern was with the identification and control of the few major determinants of human cancer (or, in an industrial context, the few major industrial carcinogens) rather than the large multitude of sins which Epstein denounces;
- if they discussed the costs and benefits of research into the control of toxic substances in the context of other possible ways of improving longevity (bad diet and tobacco in rich countries, malnutrition and infective and parasitic diseases elsewhere);
- if they took the costs of imposing restrictions on society more seriously. Epstein's general assertion is that the total direct and indirect costs of failing to regulate usually exceed the real costs of the testing and regulation, but I suspect that this is a slogan to avoid political embarrassment rather than a carefully researched conclusion;
- if they accepted that for most toxic chemicals we have both qualitative and vast quantitative uncertainty about the health benefits of restriction.

Particularly, even if the sort of grossly biased interpretation which Epstein applied to the animal saccharin data is avoided, the results of animal experiments simply are not an uncomplicated key to human hazard identification. First, moderate alteration of gross aspects of the diet of animals greatly modifies their spontaneous tumour onset rates¹¹ (so, for example, increasing the sugar content of rat diets may increase their cancer rates

more than the equivalent amount of dietary saccharin would have done¹¹). Also, any chemical which causes proliferation or necrosis in any organ that is subject to spontaneous cancers is likely to modify the onset rate of tumours in that organ, and since the aim of most animal experiments is to study a dose which is nearly, but not quite, sufficient to cause significant weight loss or mortality within three months, it is not surprising that so many chemicals at such doses can cause cancer in animals.

However, it may be that where adversary politics operate one needs views at both extremes in order to get a balanced outcome. And there is a possibility that over-emphasis on the avoidance of scientific error would emasculate the environmentalist passions and merely lead to my own rather inactive conservatism. (After all, no scruples about scientific certainty of safety usually precede the widespread introduction of new chemicals, so should the imposition of prudent restrictions require absolute proof?)

Environmentalists with biased judgments and a quasireligious certainty of right will fight more battles than any reasonable sceptic would do, and even if their victories confer 10 or 100 times less benefit on humanity than they imagine, they will in the long run probably do more good than harm — unless they materially reduce food production, distort research priorities, or direct attention away from the smoking problem in the process. Epstein justly quotes, from the 1st century AD, the Plutarch chronicles: "He who in time of faction takes neither side shall be disenfranchised". So, I feel, should both environmentalists and industrialists who suppress or deliberately misinterpret data, but they probably won't be. For the moment, the 'politics of cancer' is dominated on both sides by exaggeration. □

'For most toxic chemicals we now have both qualitative and quantitative uncertainty about the health benefits of restriction'

implement, but in the long run we do also still need to discover the main preventable causes of cancer other than smoking. Bearing both these needs in mind, there are limits beyond which it is not even politically wise to distort the science of cancer, and some of today's exaggerations may well transgress those limits. Reliable reviews of the known and suspected causes^{17,18,19} of human cancer and of trends in British

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CORRESPONDENCE

Liberties with energy

SIR,—Gerald Leach (*Nature*, 21 February, page 714), suggests that the pursuit of a high conservation/low energy strategy would not touch personal liberties. No doubt this can be so, but if this policy is maintained it is difficult to believe that a really major effect on fuel conservation is likely to be achieved. There have been many cases in industry in which fuel conservation has actually been of financial advantage to the firms concerned, but it seems likely that the proportion of firms for which this is true will decrease as time goes on, and that many firms will find the value marginal if not negative.

In a depressed and highly competitive period it is very unlikely that firms will at all readily undertake extra expenditure which is not immediately productive, and firms exerting freedom of choice are likely to stay as they are. The likelihood therefore that we shall do too little too late on a voluntary basis is really quite large, in which case Gerald Leach himself says that energy conservation may have to be forced through by controls on individuals.

I am not myself too much bothered by this. The interference with civil liberties would be little more than the controls exerted by fire, smoke control and town-planning regulations. At the same time, I can see no reason to suppose that any protection of nuclear installations that may be necessary should have a worse effect on civil liberties than the similarly necessary protection of many industrial activities such as chlorine production or the manufacture of nylon precursors at Flixborough. Freedom to walk into other people's factories does not come high on my list of vital freedoms, whether they are processing nuclear wastes or military weapons or indeed cigarettes. A severe shortage of power with strict rationing would seem to me a more serious limitation of my liberties than would the fact that I could not get into a nuclear processing works without passing somebody carrying a revolver.

If there is a genuine risk of a terrorist attempt to kill many thousands of people in a single major incident, we should have to introduce a great many controls much more onerous than the guarding of a few well defined nuclear establishments in limited areas. One would, for example, need to control an appreciable area of South Essex and North Kent to ensure that a few saboteurs with a rocket launcher should not blow up the Canvey Island stores of liquified natural gas some dark night with a westerly breeze.

Yours faithfully,

J. H. FREMLIN

Department of Physics,
University of Birmingham

CEGB on radiation

SIR,—Further to the item headed 'UK Radiation Study Overestimates Safety' (14 February, page 614). I wish to point out that the radiation dose data supplied by the Central Electricity Generating Board to the Health and Safety Executive and also published elsewhere does include the doses received by workers other than those directly employed by the CEGB. We consider it important to include the radiation doses of all classified workers in the dose data relevant to the operation of CEGB nuclear power stations. Only by using such data can sensible comparisons be made between the doses received at nuclear power stations in the UK and those in other countries.

You may be interested to know that the total dose received by non-CEGB classified persons at all CEGB nuclear power stations in

1978 was 236 man-rem, and that received by CEGB personnel was 1399 man-rem. The mean dose per man for each category was 168 mrem and 257 mrem respectively.

Yours faithfully,

R. B. PEPPER

Health and Safety Department, CEGB,
London, UK

Lost in translation?

SIR,—Your readers may know that Nobel Prize lectures are translated into many languages. Mine, delivered in 1977, described the growth of our understanding of non-crystalline semiconductors and contains the following sentence. "The discovery of this property of glasses" (namely that they cannot be doped) "certainly makes Kolomiets" (Leningrad) "one of the fathers of the branch of science that I am describing, as were others in Eastern European countries, notably Grigorovici in Bucarest and Tauc in Prague." Grigorovici remains in Bucarest, I believe retired; Tauc is in America.

For reasons unknown to me, the translated version published by the Czechoslovak Journal of Physics, with the permission of the Nobel Foundation, omits the last two of these names. A letter to the editor-in-chief from the Nobel Foundation, dated 7 November 1979 and asking for an explanation has not yet been answered.

Yours faithfully,

NEVILL MOTT

Department of Physics, University of
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Insignificant figures

SIR,—Uncritical recording of estimations with numbers that include insignificant figures is a plague in scientific literature. Although technological advancements make possible increasingly more accurate measurements, overall precision is limited by the less accurate step involved. Moreover, modern calculators tend to increase the number of figures without any relation to significance.

To quantify this abuse we have examined a selection of first class journals looking for clearly insignificant figures. The results exceeded our expectations: in the issue(s) corresponding to one month of 1979 all journals examined had several papers with insignificant figures. The journals are: *Eur. J. Biochem.*, *J. Biol. Chem.*, *Nature*, *Proc. Natl. Acad. Sci. USA*, and *Science*. For illustration, five examples taken from the above sampling are listed below (followed by appropriately rounded numbers in brackets):

enzyme activity: 913,856 U (914×10^3)
enzyme activation: $1005 \pm 80\%$ of control (10
fold activation ± 1)

glycogen in muscle: $22.43 \pm 2.89 \mu\text{mol/g}$
($22.4 \pm 2.9 \mu\text{mol/g}$)

one type of cell in mice spleen:

$104,662 \pm 18,937$ ($105 \times 10^3 \pm 19 \times 10^3$)

weight of fed female ticks: 297.26 ± 63.6 mg
(297 ± 64 mg)

From extrapolation of this small sample it seems that over 10% of currently published scientific papers contain insignificant figures.

To obtain useful information from these multifigured numbers the reader has to do some mental operations (even if largely unconsciously), to eliminate from consideration the irrelevant figures, keeping three at the most. Would it not be much better to weed out the inordinately large numbers once and for all from (computer) calculation to publication? Insignificance usually runs parallel to irrelevance. Results should be both analytically significant and scientifically relevant; everything else would be noise. For instance, the liver of a young rat may weigh in

a balance as 6.2345 g; but because of the uncertainties on how much is really "the liver" (vascular pedicle, blood, and other factors), only 6.23 g would be relevant in most cases. Four or more figures would generally be mere pseudoprecision, even if analytically significant.

The rampant pseudoprecision of most results given with 4 to 6 figures must be stopped. It is suggested that authors should carefully consider the significance of the results so that only the last given figure is tentative. This practice will usually limit, in the presentation of results, the number of figures to 3, and in many cases to 2 or even 1. Obviously this norm does not apply in the case of serial numbers, dates, etc. For arithmetic means, with non-negative data, if the coefficient of variation ($\sigma/m \times 100$) is greater than 5, the mean should be expressed with the same number of significant figures as the individual data, since such a dispersion does not justify, in general, a greater precision. Only if it is smaller than 5, an additional significant figure could be appropriate. Exponential forms (i.e. 914×10^3) will aid and simplify in most cases the final presentation of results with a number of figures limited to the significant ones.

Finally we propose to the editors of scientific journals to include in the instructions to authors a statement that manuscripts with obviously insignificant figures would not be accepted, perhaps adding that manuscripts with results with more than 3 figures should have an explicit (and acceptable) justification in the text or in the accompanying letter to the editor. Scientific objectivity would benefit, and the work of the scientists would be easier. Implicitly, this policy would recognise the fact that some scientists spend months or even years improving conditions to add one significant figure to a constant. Moreover, scientific publishing would be a little less expensive, nowadays a not insignificant consideration.

Yours faithfully,

ALBERTO SOLS

JOSÉ LUIS CARRASCO

Autonomous University of Madrid, Spain

Exhibitionism

SIR,—Your correspondent Jim Ritter, in an otherwise perceptive feature (3 January, page 6) on the proposed French science museum at La Villette, displays his ignorance of what contemporary museums have been doing during the last two decades. Far from being sources of magical effects — 'black boxes with pushbuttons' — they have developed a vast array of exhibits and programmes that make science more understandable and accessible to public visitors. Modern science museums demystify science and technology and suggest that, although complex, science can be understood, controlled, and even enjoyed.

The Association of Science-Technology Centers, to which more than 100 museums belong, would be glad to provide an itinerary of museum travels on several continents to bring him up to date.

I would recommend a tour through the tactile gallery and the visual perception exhibits of the Exploratorium (San Francisco), the human reproduction and sexuality exhibit at the Ontario Science Centre (Toronto), the plumbing exhibit and the *Food for Life* exhibit at the Museum of Science and Industry (Chicago), or the exhibition *It's About time* at the Franklin Institute (Philadelphia).

Your faithfully,

MICHAEL TEMPLETON

Association of Science-Technology
Centers, Washington DC, US

A closer look at Saturn's magnetosphere

from S. W. H. Cowley

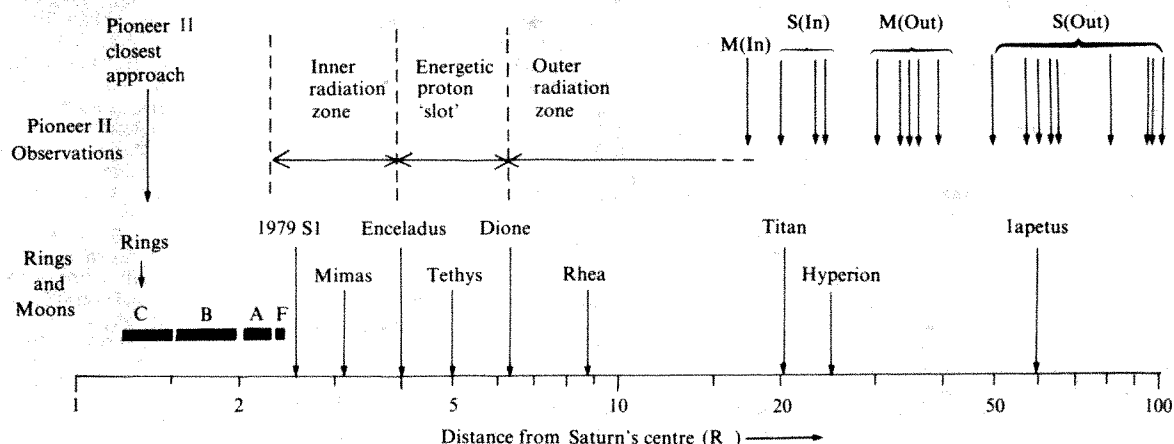
At the beginning of September, 1979 US spacecraft Pioneer 11 undertook the first close survey of the planet Saturn, approaching within 21,000 km of the visible cloud-tops. Pioneer 11 was launched in April, 1973 and had previously made the second Jupiter fly-by in December, 1974, one year after Pioneer 10. All eleven of the spacecraft's instruments which had been operating at Jupiter were still functioning 5 years later at Saturn, and sent back data on the planetary magnetic field and charged particle environment, as well as observations of the planet and its rings and moons in the ultraviolet, visible and infrared bands. A recent issue of *Science* (207; 1980) was devoted to the Pioneer results and here I shall concentrate on the discoveries made by the particle and magnetic field detectors on Pioneer (see Wolfe *et al.*, Smith *et al.*, Simpson *et al.*, Van Allen *et al.*, Trainor *et al.*, Filius *et al.*, Gehrels *et al.* and Acuna & Ness in that issue of *Science*). These included the fourth magnetosphere in the Solar System (after

Earth, Jupiter and Mercury), a new Saturnian ring and at least one new moon.

Saturn had been suspected for some time to possess a strong dipolar magnetic field, such that the planet would be surrounded by a large cavity in the solar wind plasma (which pervades the Solar System) containing the planetary field — a magnetosphere. In 1975 radio bursts from the planet, characteristic of magnetospheric plasma processes, had been detected and an equatorial field strength of ~ 1 gauss inferred (compared with ~ 0.3 gauss at Earth and ~ 4.1 gauss at Jupiter), a value which agreed well with deductions from an empirical 'magnetic Bode's law' which relates the magnetic dipole moment to the planet's spin angular momentum. Using ~ 1 gauss for the surface field strength it was estimated that the magnetosphere would extend to $\sim 40 R_S$ (R_S = Saturn's radius = 60,000 km) on the dayside, the boundary (magnetopause) location being determined by the point where the solar wind dynamic pressure and

the planetary magnetic pressure just balance. The magnetosphere would then always incorporate both the rings of Saturn and all the moons out to Hyperion, as indicated in the accompanying figure.

Pioneer 11 approached Saturn very nearly along the noon meridian (along the Saturn-Sun line), but did not in fact enter the magnetosphere until it was $\sim 17 R_S$ from the planet, as indicated by M(In) in the figure, less than half the expected distance. Subsequent magnetic measurements soon revealed the primary cause to be a rather weaker planetary field than anticipated. The equatorial surface field is ~ 0.2 gauss, with the same polarity as Jupiter's field, but opposite to that of the Earth and Mercury. In swinging round Saturn, nearly in the equatorial plane, Pioneer 11's trajectory was deflected through nearly 90° , and the spacecraft moved out from the planet nearly along the dawn meridian. In this direction the magnetopause is expected to be at a rather larger distance than at noon, and due to



Logarithmic scale plot of the position of Saturn's rings and moons, and their relationship to Pioneer 11 observations. The new F ring and moon (1979 S1) have been included, but further small moons may exist between the orbits of Mimas and the outer edge of the A ring. The D ring inside the C ring, reported from Earth observations, apparently does not exist, and no definite detection of a tenuous E ring extending out to 5 or 6 R_S was made. The moon Phoebe at $216 R_S$ has been omitted. Symbol M refers to the magnetopause (the outer boundary of the magnetosphere) observed during the noon inbound (In) and dawn outbound (Out) passes of Pioneer 11, while S indicates detection of an upstream bow shock in the solar wind.

boundary fluctuations five crossings were observed between 30 and 40 R_S . In all, Pioneer 11 spent some 2½ days within Saturn's magnetosphere.

A most surprising aspect of the magnetic data is that Saturn's field shows no detectable periodicity at the planetary spin period (~10 h), implying that the dipole moment vector is aligned within ~1° of the spin axis. This observation sets an important new constraint on dynamo theories of the origin of planetary fields, as it had generally been supposed on the basis of the other planets that a tilt angle of ~10° might be fundamental. It also frustrates attempts to measure accurately the internal rotation period of the planet.

Properties of the magnetosphere

In general, the structure and properties of magnetospheric plasmas depend very strongly on the nature of the plasma flow within the magnetosphere. Simple estimates before the Pioneer encounter showed that the overwhelmingly dominant flow in the case of Saturn (and Jupiter) should be simple corotation with the planet. This is unlike the Earth's magnetosphere, where a solar-wind driven convection rather like that in a falling raindrop is usually dominant, and where only a rather small 'core' of dipolar field lines near the Earth corotate with the planet. The difference arises mainly from the much higher dipole moments of Jupiter and Saturn compared with Earth, combined with their faster rotation rates.

In a corotating magnetosphere the only direct source of plasma is cold ions and electrons from the planet's ionosphere, together possibly with a lunar contribution if any emitting moons happen to lie within it (for example the volcanic moon Io in Jupiter's magnetosphere). This thermal plasma will be the dominant component in terms of number density inside such magnetospheres. However, more energetic charged particles originating mainly in the solar wind can become trapped in the outer part of the magnetic field if the flow fluctuates, as is always the case in practice, and the fluctuations will then diffuse these particles deep into the corotating region. An equilibrium energetic particle radiation belt will then be formed inside the magnetosphere (like the Van Allen belt inside the Earth's corotating 'core'), in which diffusion steadily feeds particles inwards from the outer source into the internal sinks (for example, absorption by the planetary atmosphere, or by moons and ring material).

The Pioneer spacecraft were not adequately instrumented to measure the dominant thermal plasma component with particle energies in the eV to keV range, but some information can be extracted from

the solar wind instrument under favourable circumstances. At Saturn, corotating thermal plasma was nearly continuously observed by Pioneer 11 during the inbound pass and distinct flux peaks were observed at the distances corresponding to the orbits of Dione and Tethys. The ionic component of this plasma appears to be mainly O^+ (or possibly OH^+) such that the source may be sputtered or photodissociated ices originating at these bodies.

Much more information is available on the energetic radiation belt particles, measurements by four instrument packages being made in the energy range from several tens of keV up to several tens of MeV. The flux of protons at fixed energy initially increases steeply with decreasing distance from the planet (as expected from inward diffusion, since particles gain energy proportional to the field strength as they move inwards). However, a peak is reached at ~7 R_S and then fluxes fall precipitately by more than a factor of 100, reaching a minimum value at the orbit of Enceladus at ~4 R_S . Absorption by Dione, Tethys and Enceladus (see figure) may be significant in forming this proton 'slot', but particle precipitation into Saturn's atmosphere due to scattering by waves excited in the thermal plasma may have a major role.

Inside the orbit of Enceladus energetic proton fluxes again rapidly rise with decreasing distance, reach a secondary peak at ~2.7 R_S before finally falling by more than three orders of magnitude to below detectability at the outer edge of the A ring. Within this inner zone of proton fluxes deep and narrow absorption features occur at the orbit of Mimas at 3.09 R_S and also at 2.52 R_S , corresponding to a previously unknown moon, designated 1979 S1, which had been detected a few hours previously on Pioneer 11 images. In addition, absorption features associated with the F ring (also newly seen on these images) were observed between 2.34 and 2.37 R_S , together with a flux plateau at 2.81 R_S which may correspond to absorption by a rather smaller new moon. The proton flux increases seen on the inside of these absorption bands seem too sharp to be consistent with the simple inward diffusion picture, and it is likely that a second source of energetic protons contributes to the formation of the inner zone, possibly the debris of cosmic ray interactions with Saturn's atmosphere and ring material. The energetic electrons show many features which are similar to the protons, but only the lower energy particles are lost in the 'slot' region. Fluxes above ~1 MeV continue to increase across the slot, if at a reduced rate, before falling to very low levels at and within the inner edge of the A ring. In general, the particle fluxes measured while the spacecraft passed under the rings were by far the lowest observed since the instruments had left the laboratory.

Pioneer's near miss

A final extraordinary event took place during the ~4 minutes in which Pioneer 11 crossed the 1979 S1 moon absorption band inbound to Saturn. For ~10 s a deep depression in the fluxes was observed by all instruments capable of such resolution which were then above background, implying that Pioneer 11 had actually passed through the flux tube containing the moon, avoiding collision by only a few thousand kilometres. The width of the flux 'hole' indicates a lunar radius of ~100 km. Similar sharp features were seen in the F-ring absorption zone, due either to more moons, or to non-uniform distribution of ring material. A final possible lunar-related disturbance was seen on the outbound pass at ~20 R_S when Pioneer 11 was ~6 R_S downstream from Titan (Saturn's largest moon) in the corotating flow. Large amplitude oscillations were observed in the magnetic field for about 2 h, possibly indicating a hydromagnetic wake produced by Titan. The corotation speed at these distances is ~200 km s⁻¹.

In conclusion, the Pioneer 11 mission to Saturn has provided a wealth of new information about the particle and field environment of the planet but leaves many questions only partly resolved, particularly as regards the thermal plasma and its sources, the nature of Titan's interaction with the magnetosphere and the existence of small moons inside the orbit of Mimas. We will not have to wait too long for some of the answers. Voyager 1 will make the second Saturn fly-by in November 1980, while Voyager 2 will reach the planet in August 1981, *en route* to Uranus. Meanwhile Pioneer 11 will continue on its way to become the second spacecraft to escape the Solar System, roughly in a direction opposite to that of Pioneer 10. It will be tracked and data on the interplanetary medium acquired into the middle 1980s. □



100 years ago

The work of casting the lenses of the great refracting telescope of the Paris Observatory has already begun at Feil's establishment. The founding of the flint disc has taken five days, and the annealing a full month. A like operation will soon take place for the Bishofsheim Observatory instrument.

A correspondent of *La Nature* sends that paper a photograph of a curious phenomenon met with in the cold of December last. It shows a bottle which contained a solution of nitrate of silver (1 per cent). The cork is forced out and imprisoned at the extremity of a long cylinder of ice, due to increase of the volume of the mass in freezing.

From *Nature* 21, 25 March, 500; 1880.

Monoclonal antibodies and immunity to malaria

from F.E.G. Cox

UNTIL the middle of the 1960s there had been very little success in immunising laboratory animals against malaria parasites and many workers felt that this meant that the possibility of a vaccine against human malaria was remote or even non-existent. The past decade, however, has produced abundant evidence that immunisation against these parasites in laboratory animals is not only possible but is in fact remarkably easy. This is unfortunately not really due to any major scientific breakthrough but to the judicious use of suitable combinations of strains of parasites and hosts in which the balance of the immune response is tipped in favour of the host. It is probably as difficult today to immunise random-bred strains of laboratory mice against *Plasmodium berghei* as it was 10 years ago, but avirulent parasites, such as *P. yoelii* and *P. chabaudi*, in certain strains of inbred mice are relatively easy to protect against even when the parasites have been secondarily selected for virulence.

Those working with primate malarias are even less fortunate for they have no avirulent parasites to choose from and are also limited to a small range of random-bred hosts. For the reasons outlined above it is possible to argue that many rodent malaria models are unrealistic but, nevertheless, the fact that protective immunity against any kind of malaria can be induced artificially does open the possibility that the mechanism involved might be relevant to the human disease.

In this context it is important to distinguish between the experimental host and the parasite itself. Undoubtedly man, monkeys and strains of mice differ widely in their ability to handle a complex antigen such as a malaria parasite. On the other hand, all malaria parasites are intrinsically similar in that in the life-cycle the sporozoites, injected into the blood by a mosquito, undergo a single multiplicative phase in the liver resulting in the release of merozoites that enter red blood cells to undergo a second phase of multiplication (or schizogony) resulting in further merozoites that invade fresh red cells to repeat the cycle until the host dies or mounts an effective immune response. The sexual stages of the life-cycle are largely irrelevant or peripheral to protective immunity although immunisation against gametocytes inhibits development in the mosquito (Mendis & Targett *Nature* 277, 389; 1979). In practice, many workers short-circuit the life-cycle by passaging infected blood thus eliminating the sporozoite and liver stages. Biochemical and ultrastructural studies suggest that

there are no fundamental differences between the malaria parasites of man and laboratory animals and, in view of the similarities in the life-cycles, it seems reasonable to assume that, as far as the parasite is concerned, what happens to it in an immunised mouse or monkey is likely to be similar to what happens to it in man.

The three major (and only possible) candidates for a specific vaccine are the sporozoite, the blood schizont and the merozoite. These three forms possess stage-specific antigens so the possibility of one of these inducing any immunity against the others is remote. The schizont has been widely used in experimental work but, notwithstanding the light that such studies have shed on our understanding of immunity to malaria, its practical potential is limited. This is mainly because there is considerable evidence to suggest that malaria parasites undergo some kind of antigenic variation and that any immunity produced is not only stage but also variant specific (Brown *Ciba Foundation Symposium* No. 25, 35; 1974).

This leaves the sporozoite and the merozoite, neither of which seems to be affected by the problems of antigenic variation (Cohen *Proc. R. Soc. Lond. B.* 203, 323; 1979). Sporozoites are logical targets because they are present before any clinically apparent infection. The use of irradiated sporozoites as antigens has been pioneered by Professor Ruth Nussenzweig at the New York University Medical Center and promising results have been obtained in mice, monkeys and man. The arguments against the use of sporozoites are that they probably need to develop in the liver to induce immunity and, as the protection is stage-specific, a single parasite entering and maturing in the liver after challenge will initiate an infection. Furthermore, sporozoites are at present difficult to obtain and multiple immunising doses have to be given. The alternative approach, a merozoite vaccine, has been developed by Professor Sydney Cohen and his colleagues at Guy's Hospital, London. This has also been successful in primates but the arguments against this approach are that there is no protection against the stages in the liver and that the vaccine is not effective unless administered in fairly large quantities with an adjuvant. The supply of merozoites for the preparation of antigen has been facilitated by the development of effective *in vitro* cultivation techniques (Trager & Jensen *Nature* 273, 621; 1978).

Even using the most favourable combinations of host and parasite, it is clear that all that can be done in terms of immunising animals with sporozoites or merozoites has been done and that the next stage will be to identify, isolate and purify the actual antigens involved in protection.

Biochemical techniques for the isolation of such antigens are time consuming but there is already evidence that potentially important antigens occur on the surfaces of sporozoites (Nardin & Nussenzweig *Nature* 274, 55; 1978; Aikawa *et al. J. Protozool.* 26, 273; 1979) and merozoites (Deans, Dennis & Cohen *Parasitology* 77, 333; 1978).

An obvious approach to the identification and characterisation of antigens likely to be of importance in protection is to use monoclonal antibodies produced by hybrid cells one of which has been derived from a lymphocyte actually producing antibody in an infected or immunised animal. Monoclonal antibodies to complex antigens are notoriously difficult to prepare but recently two groups of workers have reported considerable success. Yoshida, Nussenzweig, Potocnjak, Nussenzweig & Aikawa (*Science* 207, 71; 1980), working in New York, have isolated monoclonal antibodies directed against a single protein on the sporozoites of *P. berghei* in mice. This protein, which has a molecular weight of 44,000, is found on no other stage of *P. berghei* nor on the sporozoites of *P. knowlesi* or *P. cynomolgi*. Further work in progress by these workers indicates that mice bearing hybridomas are resistant to challenge with *P. berghei* sporozoites. At the Wellcome Laboratories in England, Freeman, Trejdosiewicz & Cross (this issue of *Nature*, page 366) have fused myeloma cells with spleen cells from mice immune to *P. yoelii* and produced monoclonal antibodies against the parasites in 38 out of 143 cultures. Five of these lines were expanded, one against all the stages in the blood, two against merozoites and two against infected red cells. Sera from mice carrying hybridoma lines were then injected into mice with a rising virulent *P. yoelii* infection and those against the merozoites protected the recipients from death. These sera, however, did not prevent the development of a considerable parasitaemia and were not nearly as protective as hyperimmune sera from *P. yoelii* immune mice.

Both of these sets of experiments can be criticised on the grounds that the models chosen could have been closer to the human ones and the authors would no doubt be the first to agree that these are very preliminary results indeed. They do, however, make a quantum jump in our approach to malaria immunology for they demonstrate that it is possible to differentiate between antigens that are actually targets in the immune response and those that are irrelevant. There is nothing special about the mouse malaria models and these findings can easily be applied to primate and human malarias. The way is now open for the use of monoclonal antibodies to bind particular antigens on malaria parasites that can then be freed from the antibody and used in immunization studies. It is here that the

mouse malaria model will be of less use because it depends on the particular nature of the immune response of that host rather than on the nature of the parasite.

These experiments have not shown that sporozoite vaccination is any better than merozoite vaccination nor that antibodies act exclusively, or even mainly, on merozoites. Furthermore, the failure of monoclonal antibodies directed against malaria antigens on the red cells to protect mice means that the idea that such antigens may somehow be involved in protection will have to be reinvestigated. The way ahead is clear, though, and it should soon be possible to implicate particular characterized antigens in the protective immune response and also clarify our uncertain understanding of antigenic variation. We might now have an extremely useful tool but we are still a long, long way from a realistic and acceptable vaccine. □

Death and the neurone

from Julian Lewis

THE great problems of neural development express themselves in many small mysteries. Among these is the phenomenon of motoneurone death. Vertebrate embryos generate at first an excess of motoneurons, and 50% or more of the initial set normally die, round about the time when neuromuscular connections begin to form. Why are these surplus cells produced, and what causes their death?

The phenomena are well-documented for several systems, chiefly in birds and amphibians, including the spinal motoneurons that innervate the limbs, the trochlear motoneurons that innervate the superior oblique muscle of the eye, and the parasympathetic motoneurons of the ciliary ganglion, which innervate the intrinsic eye muscles (for a recent review see Jacobson *Developmental Neurobiology*, 2nd ed., Plenum, 1978). The extent of motoneurone death can be modified in various ways. For example, the embryonic rudiment of a limb, either of a bird or of an amphibian, can be cut off at an early stage, before it has become innervated. This does not upset the generation of normal numbers of motoneurons in the spinal cord, nor the initial outgrowth of their axons; but it prevents their survival. Almost all the cells which should have innervated the absent limb die, and they die at about the time when motoneurone death would normally occur. The interpretation seems obvious: motoneurons survive only in proportion to the quantity of target tissue; muscles will accept only a limited density of innervation, and death is the fate

of motoneurons which fail in a competition to form synapses (Hamburger *J. comp. Neurol.* **160**, 535; 1975). Individuals of a species vary in their bodily proportions. The normal surplus of motoneurons would guarantee that there would be enough to innervate even limbs that were disproportionately large; cell death would dispose of any excess. Thus the final number of motoneurons would be matched to the amount of muscle.

This tempting suggestion is supported to some extent by another experiment. When an additional limb is grafted onto an embryo, fewer of the motoneurons die. The number saved from death is, however, less than the extra limb might be expected to maintain (Hollyday & Hamburger *J. comp. Neurol.* **170**, 311; 1976; Lamb *J. Embryol. exp. Morphol.* **49**, 13; 1979). Studies of the chick ciliary ganglion give other grounds for caution. There too, about half the motoneurons die during normal development, and almost all die if the early eye rudiment has been removed. But the cells that die suffer different sorts of death in the two circumstances. The normal death involves, for example, a swelling of the cisternae of the endoplasmic reticulum without detachment of ribosomes from it, whereas the death by peripheral deprivation involves detachment of ribosome (Pilar & Landmesser *J. Cell Biol.* **68**, 339; 1976).

In this issue of *Nature* (page 347), Lamb gives strong direct evidence against the idea that normal motoneurone death is due to competition for a target that will accept only a limited density of innervation. Lamb amputates one hindlimb bud of a *Xenopus* tadpole, and manages to divert the nerves that should have innervated it into the remaining hindlimb, which is thus supplied from both sides of the spinal cord. The proportion of motoneurons that die in this case turns out to be practically the same as normal, so that the total number of survivors innervating the single remaining limb is twice as great as normal. There is no sign of competition between the motoneurons for a limited territory; the limb has parking space to spare.

Another popular interpretation of motoneurone death is that it serves to eliminate errors in the pattern of connections. This idea also has its problems. In the normal course of development, even the motoneurons that will die send axons out into the periphery and, in chicks at least, they appear to send them to the right places in the periphery. This can be shown by tracing projections with horseradish peroxidase. Particular muscles turn out to be innervated by pools of motoneurons lying in distinctive and reproducible positions in the spinal cord; in the chick, this pattern is accurately established before the period of motoneurone death, and changes hardly at all thereafter (Landmesser *J. Physiol. Lond.* **284**, 391; 1978) although in *Xenopus* the projections shift somewhat at

early stages (Lamb *Devl Biol.* **54**, 82; 1976).

The most intriguing clue to the puzzle of motoneurone death comes from experiments with toxins that block neuromuscular transmission (Pittman & Oppenheim *J. comp. Neurol.* **187**, 425; 1979; Laing & Prestige *J. Physiol. Lond.* **282**, 33; 1978; Creazzo & Sohal *Expl Neurol.* **66**, 135; 1979). One might be inclined to guess that such toxins should cause, if anything, increased death of motoneurons, mimicking the effect of absence of muscle. Precisely the opposite is observed: curare, α -bungarotoxin, α -cobratoxin and botulinum toxin, given in the period when motoneurons normally die, can all prevent the death. In this they are effective so long as movement is paralysed; when the concentration of toxin falls to the point where movements can begin, the surplus motoneurons belatedly die. The normally-occurring cell death evidently depends on neural activity. The phenomenon must therefore be closely linked with another little mystery of neural development, concerning spontaneous movement: why is it that vertebrate embryos start to writhe and squirm and wave their limbs about haphazardly as soon as neuromuscular connections have begun to form (Hamburger in *Behavioural Embryology*, vol. 1 (ed. Gottlieb) Academic Press, 1973)?

The activity of a motoneurone depends on the inputs which it receives in the spinal cord; and it is after all the set of conditions under which a neurone fires, not the location of its soma, that matters for the functioning of the system. If, abandoning the notion that motoneurons die in a competition for a limited quantity of target tissue, we espouse instead the theory that they die because they have made mistaken connections, we must suppose that the mistakes lie not in the relation between the position of the soma and the choice of target muscle, but rather in the relation between the choice of target muscle and the set of central inputs to the motoneurone. A simple speculation along these lines might be that some motoneurons die because they are, in their time of firing, out of step with their fellows in a pool of motoneurons innervating a single muscle; they might feel the effects at their point of contact with that muscle, during periods of muscle stimulation. From a very early stage, there are indeed correlations in time of firing between pools of motoneurons innervating different muscles, and these become sharper as development proceeds (Bekoff, Stein & Hamburger *Proc. natn. Acad. Sci. U.S.A.* **72**, 1245; 1975; Bekoff *Brain Res.* **106**, 271; 1976). But again, the speculation encounters difficulties. It would suggest, for example, that where there is less scope for mistakes, there should be less death: that in a nucleus such as that of the trochlear nerve, which innervates only one muscle, there should be no confusion of connections, and all the motoneurons should survive. But they

don't. In the trochlear nucleus of a bird, just as in the ventral horn of its spinal cord, half the motoneurons die (Cowan in *Development and Aging of the Nervous System* (ed. Rockstein), Academic Press, 1973).

The evidence so far has not yielded a solution to the puzzle of motoneurone death; but it has at least proved that the solution will be interesting. □

Birds see ultraviolet light

from J.K. Bowmaker

HUMAN colour vision extends from about 400 nm in the violet to about 750 nm in the red. Why are we restricted to this limited range? About 80% of the electromagnetic radiation reaching the Earth's surface lies between 300 nm in the ultraviolet and 1,100 nm in the infrared, but nevertheless only part of this spectrum is available for vision since radiation with wavelengths longer than about 800 nm has too little energy to cause photochemical reactions. Thus there is available for vision a spectral range from about 300 nm to 800 nm. However, the limits of an animal's vision are determined by the visual pigments contained within the photopic receptors of the retina, the cones, and by pre-receptor filters. In man these are principally the lens that cuts off light below about 400 nm, and the macula pigment in the yellow spot of the retina. Filtering violet and near ultraviolet light reduces chromatic aberration which becomes increasingly severe at short wavelengths.

The question remains whether other species are similarly restricted. It is natural perhaps to compare the visual sense of other diurnal species with our own, on the assumption that we represent the culmination of the evolution of colour vision. But is this reasonable? The early mammals, around at the time of the dinosaurs, were probably nocturnal, resulting in their colour vision becoming largely degenerate, with many modern mammals having only limited dichromatic vision. The Old World primates, including man, are somewhat of an exception in that they have evolved secondarily trichromatic vision along with the adoption of diurnal habits. Even so our trichromacy has a 'blue' channel that is about 100 times less sensitive than the 'red' and 'green' channels. The true culmination of the evolution of colour vision in vertebrates would be expected to be found in highly evolved diurnal animals, perhaps best represented by diurnal birds and it is

within these species that we should look for colour vision making use of more of the available spectrum.

The most striking feature of the retina of diurnal birds is the presence of brightly coloured oil droplets contained within the cones that act as highly selective long-pass cut-off filters (see Bowmaker *Vision Res.* 17; 1129; 1979). The lenses of most diurnal birds are optically clear and it seems that one of the functions of the oil droplets is to limit chromatic aberration selectively at the receptor level. However, amongst the coloured oil droplets there are colourless ones that presumably transmit near ultraviolet light with the possibility that diurnal birds can detect these short wavelengths as a 'colour', different in hue from the rest of their visible spectrum.

It is known that pigeons can see in this spectral region (Wright *J. exp. anal. Behav.* 17, 325; 1972) and in a recent paper Timothy Goldsmith (*Science* 207, 786; 1980) has presented evidence that several species of humming bird can discriminate the near ultraviolet. Working with a natural population of the birds he set them a simple task of selecting artificial feeders containing a sugar solution on the basis of a coloured viewing screen set in the feeders. The birds were able to distinguish the near ultraviolet around 370 nm from darkness or from the small amount of far red light that leaked through the ultraviolet transmitting glass filter, which human observers were unable to do. The birds could also distinguish white light lacking wavelengths below 400 nm from white light from a quartz-halogen bulb that does contain near ultraviolet radiation.

The near ultraviolet sensitivity of the humming birds presumably involves cones containing an oil droplet that transmits short wavelengths but also a visual pigment that is sensitive to these wavelengths. Evidence from pigeons and chickens (Graf & Norren *Vision Res.* 14; 1203; 1974) indicates that in addition to red, green and blue-sensitive visual pigments there is a visual pigment with maximum sensitivity at about 400–415 nm. This is very close to the maximum sensitivity of the visual pigment of human 'blue' cones (415–420 nm) recently recorded by Bowmaker and Dartnall (*J. Physiol. Lond.* 298, 501; 1980), but the effective maximum sensitivity of the human cones is displaced to about 440 nm with a marked cut off below about 400 nm due to the filtering of the lens.

The other major group of animals known to have vision in the ultraviolet are the insects. Here chromatic aberration is not a problem since arthropod eyes are designed on different optical principles. These insects, principally bees and moths, have mouthparts designed to suck nectar from flowers, and many of the flowers rely on their insect visitors for pollination. Many such flowers reveal striking patterns of 'nectar guides' if photographed using ultraviolet sensitive film that are not visible

to a human observer and it is assumed that the evolution of such guides has paralleled the evolution of ultraviolet vision in these pollinating insects. It would be interesting to know whether the flowers usually frequented by humming birds have similar ultraviolet nectar guides. However, as Goldsmith says "the interplay of several spectral classes of oil droplet with several cone pigments and the presence of receptors functioning in the near UV suggests that avian colour vision possesses a richness beyond our ken". □

BL Lacertae Objects —the 'missing link'?

from a Correspondent

A RECENT study of a sample of extragalactic radio sources belonging to the so-called BL Lacertae class has shed some new light on one of the most interesting questions in extragalactic radio astronomy, namely the relationship between the radio sources associated with galaxies and those associated with quasars.

Until 1968 the optical objects found to be associated with extragalactic radio sources could be divided into two basic classes, galaxies and quasars. The galaxies have a variety of forms, basically appearing diffuse on photographs; their optical spectra are also varied, ranging from the thermal continua and weak absorption lines typical of old stars to the non-thermal continua and strong emission lines which are indicative of violent activity in the galactic nucleus. This latter type of spectrum is also typical of the quasar which appear starlike on photographs and are generally at higher redshifts than the galaxies. In 1968 an apparently new class of identification was found when the radio source VRO 42.22.01 was identified with the so-called 'variable star' BL Lacertae. The radio source itself was very unusual showing striking, rapid variations in radio flux density and in polarization. In this respect it bore similarities to the very compact sources associated with some quasars though its variations were more extreme. However, the optical spectrum of the 'star' was apparently completely featureless showing neither emission nor absorption lines, the continuum being clearly non-thermal in origin; the optical radiation was also strongly polarized and very variable in intensity. The absence of lines in the spectrum made this very peculiar object particularly tantalizing as there was no means by which to determine its redshift and its distance.

Subsequently many more sources of this type, now called BL Lac objects, have been found and to date roughly 60 are known.

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The defining properties of the objects in this class are that their optical spectra have steep non-thermal continua with no strong emission features, there is rapid variability at optical, radio and infrared wavelengths, and there is strong and rapidly varying polarization. The objects vary quite widely in optical appearance, ranging from those which are completely stellar to those which are the nuclei of galaxies. Though it was originally thought that the optical spectra were completely featureless, careful spectroscopic work on some of these objects, particularly BL Lac itself, has revealed weak emission and absorption features similar to those found in elliptical galaxies, so that it has been possible to determine the distances and physical properties in some cases. It is now possible therefore to compare their physical properties with those of quasars and radio galaxies and to investigate their relationship to these objects.

Such an investigation has been carried out by Weiler & Johnston (*Mon. Not. R. astr. Soc.* 190, 269; 1980) who have made a detailed radio study of a large sample of BL Lac objects, examining their radio structures on scales ranging from a few thousandths of an arc second to arc minutes. Their results indicate that the majority of the BL Lac objects contain very compact cores with at least half of the total flux density and with properties very similar to the compact variable quasars. However they also find that over half of them have extended radio emission as well, with sizes and luminosities comparable with those of the extended sources associated with radio galaxies and quasars; the BL Lac objects are thus apparently indistinguishable from the radio galaxies and quasars on the basis of their radio structures. Furthermore, as the luminosities of their compact radio cores span the whole range from the relatively weak cores seen in radio galaxies to the stronger ones in quasars, they seem to form a 'link' between the quasars and radio galaxies. Further evidence of such a link comes from the fact that the BL Lac objects appear to occupy an area intermediate between that covered by quasars and that occupied by radio galaxies on a radio flux density-optical magnitude diagram.

It thus seems possible that the BL Lac objects represent a transition phase between quasars and radio galaxies. Weiler and Johnston speculate that a radio quasar may represent the early stages in the evolution of an elliptical galaxy which contains a significant amount of gas in its central regions; this gas, as well as producing the observed emission lines, is used in some way to power the central 'engine' which is responsible for the violent radio and optical activity. If this gas is used up or expelled the emission lines will disappear and the operation of the engine may become more variable, so that the quasar may evolve into a BL Lac object.

Finally as all the gas is used up, the activity may decrease still further leaving the relatively weak core sources seen in most radio galaxies.

Although this evolutionary sequence is not the only interpretation of the results it is certainly an interesting possibility. There is much scope for further work to clarify the situation. It is important to determine the space densities of the BL Lac objects to test that these are consistent on the basis of the evolutionary sequence with the already known space densities of quasars and radio galaxies. However, this analysis may be difficult if only because unambiguous selection of a sample of BL Lac objects is almost impossible as a result of the degeneration in the definition of their properties and the continuity of these properties with those of radio galaxies and quasars. More insight is likely to be gained from detailed information on the radio structures of the BL Lac objects on all angular scales; in particular comparison of the structures of the BL Lac cores with those of radio galaxies and quasars on milliarcsecond scales will be particularly revealing as it is these core sources that provide perhaps the strongest evidence for the evolutionary sequence. □

Towards the ideal seismic experiment

from Bob Whitmarsh

THE most powerful remote sensing technique used in the search for hydrocarbons is seismic reflection. Carefully controlled sources radiate acoustic energy which is reflected from sub-surface interfaces and sensed by a large number of transducers usually arranged in a linear array relatively close to, and aligned with, the source. Subsequently complex and highly sophisticated signal processing techniques are applied to the recorded signals to increase the depth range or penetration of the final reflection profile as well as its vertical and horizontal resolution. The ultimate objective of the reflection technique is to translate the observed vertical reflection times from different strata into depths and this requires a knowledge of seismic velocities in the overlying layers. During a normal reflection survey the accuracy of the velocity determinations is limited, particularly for deeper reflectors, by the length of the receiving array. This length determines the range of incident angles at the reflector for the group of rays from a single shot.

More accurate information about the deeper velocity structure can be obtained if

the distance between source and receiver is steadily changed and this type of experiment, radically different from normal reflection profiling, can be used to study reflections from vertical incidence out to the ranges where critical reflection occurs and beyond. At sea, until recently, such variable angle reflection experiments have mostly been carried out using one or more free-drifting surface sonobuoys or fixed ocean-bottom seismographs. The optimum procedure for reflection studies however is to use a source and receiver which move with equal but opposite velocities away from a fixed point (the common depth point (CDP)). Then all the reflections picked up by a towed array from a given interface originate at the same fixed area on the interface irrespective of the incident angle thereby avoiding the difficulties due to irregular interfaces. At sea the only practical solution is to use two ships, it being a great advantage if one of these is fitted with a multichannel hydrophone array. This is the procedure recently reported by Stoffa & Buhl (*J. geophys. Res.* 84, 7645; 1979) which they have used to carry out a series of deep crustal studies of oceanic areas.

The first description of a two-ship multichannel seismic experiment to study oceanic crust seems to have been that of Limond *et al.* (*Earth planet. Sci. Lett.* 15, 361; 1972). They fired a 34-km long expanding spread profile (ESP) in the Bay of Biscay. Shots fired every 800 m were received by a 2.4 km long 24-channel array thereby providing three-fold coverage. As the object was to obtain Moho reflections on the multichannel reflection profile (these were identified by working back from Moho refracted arrivals through sub-critical reflections to vertical incidence) the signal processing of the seismic traces did not proceed beyond the presentation of a record section.

The experiments of Stoffa and Buhl however were specifically designed to study the deep oceanic crust with the greatest practical precision. In such experiments both horizontal range and shot-to-receiver travel time need to be determined to within a few tens of metres and a few milliseconds respectively. Ideally, precise absolute navigation is required so that the two ships lie symmetrically about the common depth point at all times. In practice, even though the relative navigation of the ships is correct, as can be established using a ship-to-ship radio ranging system, surface currents (which may not be detectable during the experiment) can introduce important errors. Such errors probably exceed those due to dipping reflectors. Their combined effect tends to invalidate the basic assumption underlying CDP but fortunately does not necessarily negate the usefulness of ESP experiments. Except in nearshore areas covered by high quality radio-navigation systems there is at present no simple practical solution to this navi-

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gational problem. The timing precision is easier to obtain, particularly with electrically fired airguns or explosions.

In recent years advances in marine seismic work have come more from new methods of quantitative analysis and numerical modelling than of experimental technique. An example of such an analytical method is that used by Stoffa and Buhl to extract phase velocity information from the record sections. This is done on a shot by shot basis using a semblance technique to determine the phase velocity having the greatest coherence across each set of 24 traces beginning in turn at each successive digitised sample along the nearest or first trace. The technique is useful in that it enables over-lapping or 'crossing-over' signals of different phase velocity to be separated as well as giving an objective assessment of the onset times of second arrivals which might otherwise have been picked too late. From analysis of the semblance plots a precise travel time *versus* distance plot is produced for all arrivals whose semblance coefficient exceeds a given value, and this plot in turn is inverted using a derivation of a standard method to give a highly detailed velocity/depth profile of the oceanic crust.

A disadvantage of the ESP method outlined above is that the structure under each shot and receiver position may differ, whereas for a fixed ocean-bottom seismograph at least half this possible lateral variation is avoided. However some indication of lateral velocity inhomogeneity can be obtained, again using two ships, by keeping the source and receiver at a fixed distance (constant offset profile or COP) appropriate to the interface of interest. By combining COPs having different offset distances with CDP and normal multichannel reflection profiles it is possible to approach the ideal deep crustal seismic experiment. However to model the Earth fully taking account of all the constraints provided by these types of observations is probably taking the problem beyond the scope of the best computer facilities.

The technique described by Stoffa and Buhl opens up exciting possibilities for detailed deep crustal studies in the oceans. In practice however it is unlikely that there will be many researchers in a position to follow their example. Regrettably, multi-channel digital recording systems, digital seismic reflection processing and two-ship experiments are expensive. But such experiments might continue perhaps on an inter-institution basis in areas of mutual scientific interest. Continental margins in particular, where the results could be of more than academic interest and where two-ship experiments are more likely to obtain the necessary funding would provide attractive targets. Such an approach has already led to a joint French-United Kingdom study of the margin southwest of the English Channel last year.

Recent advances in polarized electron sources

IN his article "Polarized electrons are going around in circles" (*News & Views* 283, 248; 1980) C.B. Lucas notes that recent advances in sources of polarized electrons should provide insights into fields as diverse as neutral currents, surface physics, and molecular optical activity. He calls attention to recent developments in atomic physics-based polarized electron sources and to the fact that beams of electrons and positrons become polarized as they circulate in a storage ring. We would like to describe some very recent advances that have been made in the development and application of solid state sources of polarized electrons.

Extraction of electrons from a ferromagnetic solid offers a conceptually elegant source of polarized electrons. The electrons are aligned by an external magnetic field and exist in densities unobtainable in aligned atom beams. A very useful source has been demonstrated¹ using field emission from a tungsten tip that has been coated with europium sulphide. At the operating temperature of 10 K, the EuS is ferromagnetic and acts as a spin filter. A polarization of 0.85 is obtained at currents up to 10 nA. Most notably, the source has very high electron optical brightness and will be useful in applications requiring highly collimated, highly focused beams.

The most recently developed² solid state source of polarized electrons uses photoemission from gallium arsenide. When GaAs is irradiated with circularly polarized light, the electrons excited from the maximum of the spin-orbit-split valence band may be polarized up to a theoretical value of 50%. GaAs is one of those special materials in which, by application of caesium and oxygen to the surface, the vacuum level can be lowered below the bulk conduction band minimum to produce the so-called 'negative electron affinity' emitter. Negative electron affinity GaAs is not only an extremely efficient photoemitter but the electrons can be polarized! When the light is modulated from right to left circular polarization, the direction of the polarization of the electron beam is also modulated, facilitating the use of very sensitive signal detection techniques.

A source of polarized electrons utilizing GaAs has been built which produces a continuous beam with a polarization of $\pm 43\%$, and a beam

current of 20 μA has been obtained for one milliwatt of incident circularly polarized light². A pulsed GaAs source has produced microsecond pulses of several hundred milliamperes at a 120 Hz repetition rate³.

In a sense, the GaAs source is the solid state analogue of the source utilizing the 'Fano effect' in caesium discussed by Lucas. The higher density of the solid provides much more intense electron beams; typically three orders of magnitude more intense than existing Fano effect sources. The energy spread of the GaAs source is 130 meV FWHM, and the effective source area is determined by the focus of the circularly polarized light, typically 0.5 mm diameter. The electron optical brightness is second only to the EuS/W field emission source.

The Fano effect in Cs can produce electron beams nearly 100% polarized. In most experiments the lower polarization of the GaAs source is easily compensated by increasing the intensity of the beam, but there are experiments, for example, where target damage is a problem, where higher polarization is required. There is hope of increasing the polarization of the GaAs source by lifting the degeneracy at the valence band maximum by (1) stressing the crystal (2) preparing epitaxially grown multilayer structures to confine the electron in one-dimensional potential wells, or by using (3) crystal field splitting in chalcopyrite semiconductors such as CdSiAs_2 or CdGeP_2 , which are ternary analogues to GaAs.

The GaAs source has already been very effectively applied at SLAC to measure the parity non-conservation in inelastic scattering of 20 GeV polarized electrons from hydrogen and deuterium³. It has been used to observe the surface magnetization of ferromagnetic nickel through the exchange interaction in low energy polarized electron scattering experiments⁴. It has also been used in polarized electron diffraction studies of single crystal tungsten⁵. The recent demonstrations of the characteristics of this type of source have led to the construction of such devices by nearly every group engaged in polarized electron scattering.

We agree completely with Lucas that there are now many exciting research opportunities because of recent advances in polarized electron sources. We believe that the new solid state source technology will play a significant part.

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ARTICLES

The narrow rings of Jupiter, Saturn and Uranus

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Recent occultation studies and spacecraft observations have revealed the presence of narrow ring systems around the planets Jupiter, Saturn and Uranus. Thus three of the four major planets are now known to contain systems of narrow rings. This article attempts to account for the origin and location of these rings.

OUR knowledge of the uranian rings is extensive and it is possible to list a large number of features which any theory of narrow rings ought to be able to account for (see ref. 1). We know little about the jovian ring system at present, apart from its location and the fact that the outer ring is only 6,000 km wide². This ring contains a bright inner ring of width 800 ± 100 km (ref. 2). Voyager 2 images have revealed the presence of a small, dark satellite of diameter between 20 and 40 km with an albedo ~ 0.05 (refs 3, 4). The orbit of this satellite lies in the ring plane and appears to define the outer edge of the rings as seen by reflected light (ref. 4 and G. E. Danielson, personal communication).

Pioneer 11 detected at least one comparatively narrow saturnian ring and possibly several small satellites, some of which may orbit within the rings^{5,6}. The radial width of the F-ring discovered by the Pioneer 11 imaging team⁶ is not greater than 800 km (ref. 6). The 3,500-km gap between this ring and the broad A-ring (the Pioneer gap) contains less material than Cassini's division⁶. The Pioneer 11 Geiger tube team⁵ detected a possible ring of width 3,600 km between 2.550 and $2.490 R_s$ ($R_s = 60,000$ km is the equatorial radius of Saturn) which may contain two satellites of diameter ≥ 170 km at 2.534 and $2.522 R_s$. They also detected a ring (F) of width 2,100 km between 2.371 and $2.336 R_s$, which may contain two satellites at 2.343 and $2.350 R_s$, and a possible new satellite at $2.82 R_s$. The fact that the two determinations of the width of the F-ring are quite different suggests that the ring is wide ($\sim 2,100$ km) but, like the jovian ring, contains a bright inner ring of width ~ 800 km.

It is well known that the 2:1 Mimas resonance lies close to the outer edge of the B-ring⁷. We point out here that the 3:2 Mimas resonance ($2.361 R_s$) lies close to the outer edge of the F-ring ($2.371 R_s$) and that the 4:3 Mimas resonance ($2.553 R_s$) lies close to the orbits of the newly discovered satellite or satellites at 2.534 and $2.522 R_s$. These calculations assume that the coefficient of the principal gravitational harmonic $J_2 = 0.01667$ (ref. 9) but neglect the mass of the rings since this is not known.

Dermott *et al.*¹ consider that the extremely narrow widths and sharply defined edges of the uranian rings imply that some kind of critical phenomenon is responsible. They suppose that each ring contains a small satellite which maintains solid particles in stable, horseshoe orbits about its lagrangian equilibrium points (see Fig. 1). They show that this model explains most of the observed features of the uranian rings. By comparison Goldreich and Tremaine⁸ suggest that each ring consists of particles confined between the orbits of two small satellites.

They show that this theory can explain how the particles can remain in precisely aligned elliptical orbits but they do not explain other features of the rings, such as sharp edges and the narrow range of widths of the rings.

The uranian and jovian ring systems, both of which are comparatively narrow, have mean orbital radii of 1.8 planetary radii. We consider it significant that both systems also lie in the Roche 'zone' defined by Dermott *et al.*¹ (see Figs 2 and 3). The location of the narrow jovian ring suggests an origin similar to that of the uranian rings. Whether this ring consists of a number, possibly a large number, of discrete rings or whether it is continuous is not yet known. We suggest that the jovian ring is the product of the disintegration of a satellite that entered the Roche zone, probably as a result of orbital evolution due to tidal dissipation, and that large numbers of small particles are now maintained in horseshoe orbits about the lagrangian equilibrium points of the remanent chunks. The recent observation of a small satellite in the outer edge of the rings supports this view. Collisional, as well as tidal processes were probably involved in

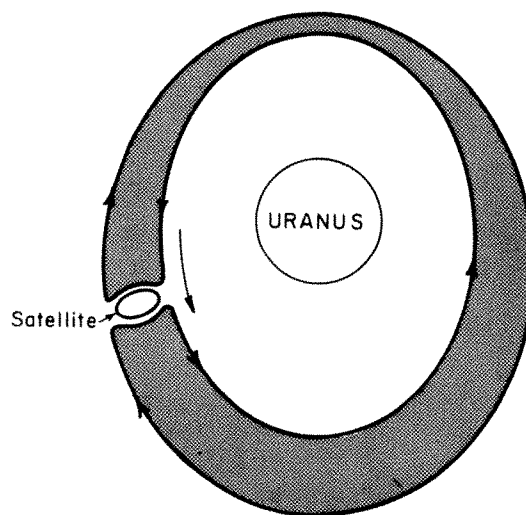


Fig. 1 Dermott *et al.*¹ suggest that each uranian ring contains a small satellite which maintains particles in stable, horseshoe orbits about its lagrangian equilibrium points. In their model, loose solid particles leave the satellite surface and enter orbits closely similar to that of the satellite (which can be both eccentric and inclined to the equatorial plane of the planet). It is the gravitational force of the satellite in a resonance with the ring particles which then provides the critical phenomenon needed to define a narrow, sharp-edged, asymmetric ring.

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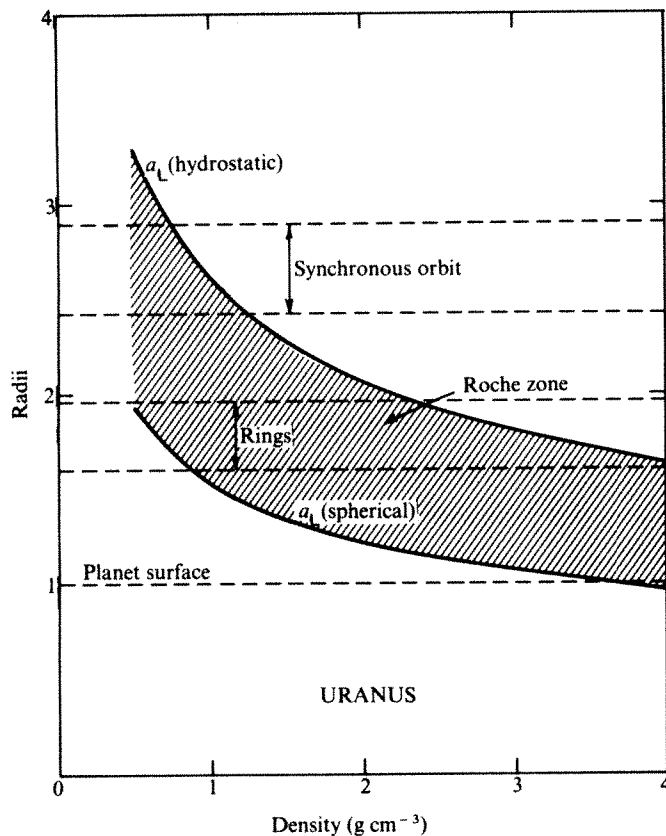


Fig. 2 Roche limit a_L for Uranus as a function of satellite density. a_L (hydrostatic) applies to a satellite that can relax to hydrostatic equilibrium and a_L (spherical) applies to a spherical satellite. Dermott *et al.*¹ refer to the zone between these two extremes as the Roche zone. It is in this zone that a satellite is expected to lose solid particles from its surface. The location of the synchronous orbit shown above is based on the rotational period of 12.8 ± 1.7 h obtained from occultation data by Elliot *et al.*¹⁰.

the break-up of the original satellite. The positions of the rings with respect to the Roche zones and the synchronous orbits suggest that the jovian ring satellites have densities $\geq 3 \text{ g cm}^{-3}$ and that the uranian ring satellites have densities $\geq 2 \text{ g cm}^{-3}$. Most of Saturn's broad ring system and the newly discovered narrow rings lie above its synchronous orbit, hence the mechanism of ring formation discussed above cannot be applied.

Particle paths

The path of a ring particle when the orbit of the ring satellite is circular can be studied by considering the Jacobi integral. For motion in horseshoe orbits it is convenient to write the Jacobi constant C as¹¹

$$C = 3 + \alpha m^{2/3} \quad (1)$$

where m is the satellite-planet mass ratio. We have found, by numerical integration of particular cases, that the type of path followed by a particle depends on the value of α . If we write

$$a_i = 1 + \Delta a_i \quad (i = 0, 1, 2) \quad (2)$$

and

$$|\Delta a_0| - |\Delta a_j| = \pm m^n \quad (j = 1, 2) \quad (3)$$

where a is the semimajor axis of the particle and the subscripts refer to the number of consecutive encounters with the ring satellite that the particle has experienced, then we find that n varies with α and that n increases to values near unity as α tends to zero. Our results for $m = 10^{-9}$ are shown in Fig. 4. We also conclude from these experiments that motion in a horseshoe is

unlikely if α exceeds about 1.2. The existence of this limit may account for the sharp edges of the rings.

For motion in a circle

$$\Delta a = 2 \left(\frac{\alpha}{3} \right)^{1/2} m^{1/3} \quad (4)$$

Thus the width of the ring is proportional to $m^{1/3}$ and a wide range of satellite masses produces a comparatively narrow range of ring widths. This could account for the observation that the uranian rings have a small spread of widths.

For small values of α (≤ 0.3), the changes in a on encounter are such that Δa_0 and Δa_1 are almost exactly equal and opposite. If we write

$$\delta a = (|\Delta a_0| - |\Delta a_1|) / \Delta a_0 \quad (5)$$

then

$$\delta a \sim \pm m^{n-1/3} \quad (6)$$

and, for $\alpha \leq 0.3$, $n \geq 2/3$ and $|\delta a| \sim m^{1/3}$. Thus, for $m \leq 10^{-9}$ (10^{-9} is greater than the satellite masses needed to account for the widths of the uranian rings) $|\delta a| \lesssim 10^{-3}$ and

$$\Delta a_0 = -\Delta a_1 \quad (7)$$

to 1 part in 10^3 or greater (see Fig. 5). It is this symmetry which accounts for the peculiar stability of very narrow rings.

This symmetry is not a property of the circular orbit case alone. If the ring satellite has an eccentric orbit, and if Δa_0 or α is small and e_0 and e_s , where subscript s refers to the ring-satellite, are not too different, then the changes in a on encounter are still symmetrical. Here, we define α by equation (4) even though the Jacobi integral does not apply to the eccentric orbit case. Our results for $m = 10^{-9}$ and $e_s = 0.01$ (which is close to the mean eccentricity of the uranian ϵ ring) are shown in Fig. 4. There seems to be no substantial difference between the circular orbit and the eccentric orbit cases.

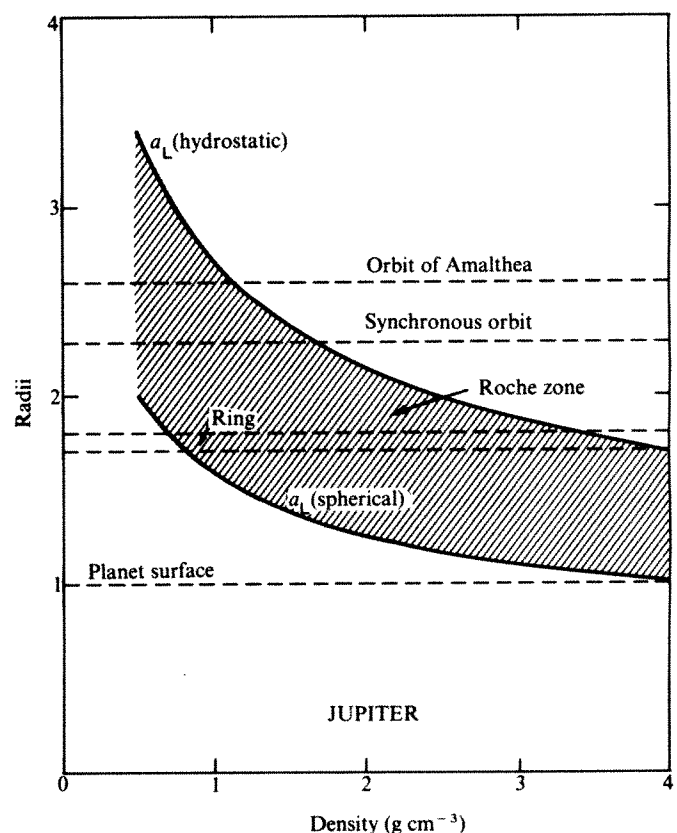


Fig. 3 Roche limit for Jupiter as a function of satellite density. Note the similarity between the location of the rings on this figure and those in Fig. 2.

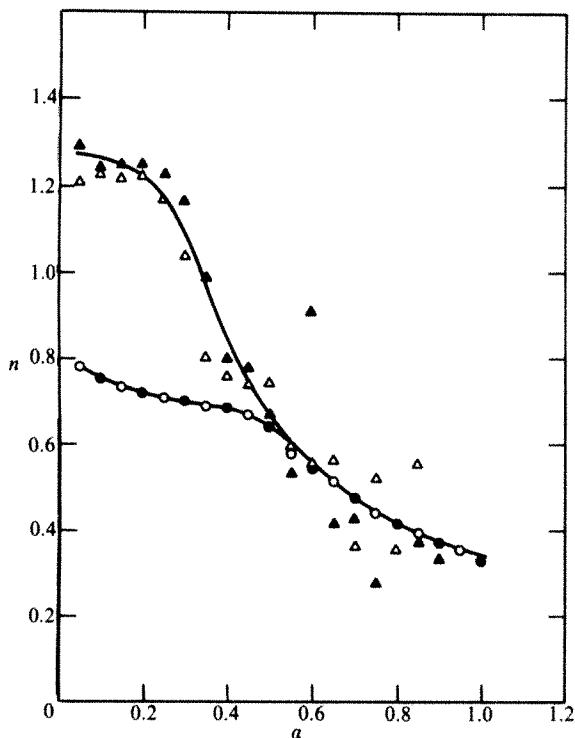


Fig. 4 n is a measure of the symmetry of the horseshoe path about the line $a = 1$ (see equation (3) and Fig. 5). The circular points refer to changes in the semimajor axis a of the ring particle orbit after a single encounter with the ring-satellite: ●, the circular orbit case ($e_s = 0$, where e_s is the eccentricity of the ring-satellite orbit); ○, the elliptical orbit case ($e_s = 0.01$). Triangles indicate the total change in a after two consecutive encounters: ▲, the circular orbit case ($e_s = 0$); △, the elliptical orbit case ($e_s = 0.01$).

The effect on the orbit of a ring particle of an external force due, for example, to Poynting–Robertson light drag is shown in Fig. 5. If α is small (≤ 0.3), then Δa_0 and Δa_1 are always equal and opposite: drag forces have little influence on this phenomenon. Therefore, if the ring is very narrow and the magnitudes of the drag forces acting on the particle on the inside and on the outside of its horseshoe path are not too different, then the orbital decay achieved in one half of the path is, in effect, cancelled by that achieved in the other. Second order effects due to the finite width of the ring are very small and we will not discuss them here.

As the drag force extracts angular momentum from the ring system, some orbital decay must occur. But the orbits of the ring particles and the ring satellite decay together at some rate r times less than that of an unconstrained ring particle.

$$r \sim \frac{m_r}{m + m_r} \quad (8)$$

where m_r is the total mass of the ring particles. For a ring of optical depth unity,

$$r \sim 5 \times 10^{-4} \left(\frac{a}{50,000 \text{ km}} \right) \left(\frac{d}{0.1 \text{ cm}} \right) \left(\frac{10 \text{ km}}{W} \right)^2 \quad (9)$$

where W is the mean width of the ring. Thus, even if the rings of Uranus consist of very small particles ($d \ll 0.1 \text{ cm}$), Poynting–Robertson drag acting over times comparable with the age of the Solar System would not result in significant orbital decay or ring spreading.

Radiation pressure acting on small (micrometre size) particles in an optically thin ring could distort the particle paths and, in the case of the jovian ring, the discussion we have given in terms of simple horseshoes may not be appropriate for these very small particles (J. A. Burns, personal communication). However, we contend that the presence of small satellites in the jovian ring is needed not only to provide a localised source of ring material,

but also to account for the partial confinement of the ring particles in a comparatively narrow lane.

Ring geometry

The remarkable variation in width of the uranian ϵ ring is a consequence of the ring's large eccentricity gradient $g(= a \, de/da)$. The ratio w of the maximum and minimum ring widths is given by¹

$$w = \frac{1+g}{1-g} \quad (10)$$

The mean ring width is $2\Delta a$, where Δa is half the difference of the semimajor axes of the ring's inner and outer edges. Since w must be positive, we must have $g < 1$. For the uranian ϵ ring $\Delta a = 30 \text{ km}$ and $g = 0.62$ (ref. 12). Thus, g is not merely non-zero, it is also close to the critical value of unity. Accounting for this phenomenon is by far the largest hurdle that any theory of the rings of Uranus has to surmount.

If a ring has a uniform eccentricity gradient and the particles in the ring have a common pericentre, then the relative velocities at pericentre are very much less than those at apocentre. We consider that it is probably this asymmetry which gives rise to the systematic changes in the orbital elements of the ring particles on encounter with the ring satellite.

Some actual changes in the orbital elements produced by encounter are shown in Fig. 6. The parameters chosen for this particular numerical experiment were $\alpha = 1.2$, which is the critical value of α for horseshoe motion, $m = 10^{-10}$ and $e_s = 0.00780$, which is the mean eccentricity of the uranian ϵ ring. If $a = 51,284 \text{ km}$, which is the mean semimajor axis of the uranian ϵ ring, then these parameters give $W = 2\Delta a = 60 \text{ km}$, which is the observed mean width of the ring. The values of a_s , e_s , a_0 and e_0 were dictated by the observed eccentricity and eccentricity gradient of the ϵ ring. Thus, particles on the outside of the ring were given values $e_0 = 0.00816$ and those on the inside values $e_0 = 0.00744$. The initial phases of the particles with respect to the satellite were chosen randomly with approximately equal numbers on the inside and on the outside of the ring.

The histogram of the values of g produced by this experiment (see Fig. 6) shows a strong positive bias with a peak at $g \approx 0.5$, which is close to the gradient ($g = 0.62$) defined by the edges of the ϵ ring. However, many factors will have an influence on this experiment. These include α , m , and, in particular, the shape of the satellite. These factors have yet to be investigated in detail. It would appear that the horseshoe orbit model of Dermott *et al.*¹ may account for the observed eccentricity gradient of the uranian ϵ ring, but we do not regard this question as settled. However, we have confirmed that the changes in pericentre $\tilde{\omega}$ of the ring particle orbit on encounter are small ($\leq 2^\circ$). Hence, as particles in horseshoe orbits spend equal amounts of time on the

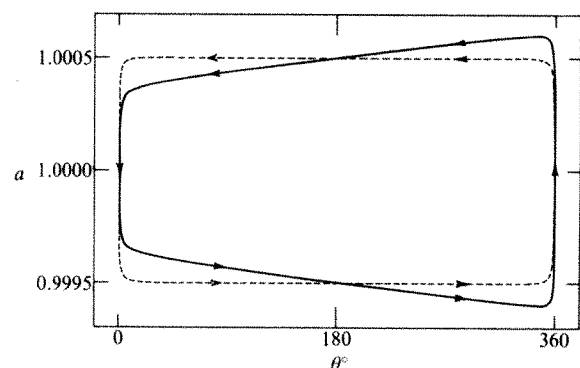


Fig. 5 Path of a particle moving in a horseshoe orbit about a ring-satellite of mass $m = 10^{-9}$ (circular orbit case). Semimajor axis a is plotted against the angular separation θ of the particle and the satellite. The dashed line refers to the particle path in the absence of drag and the solid line shows the effect of an external drag force. Both paths are symmetrical about the line $a = 1$.

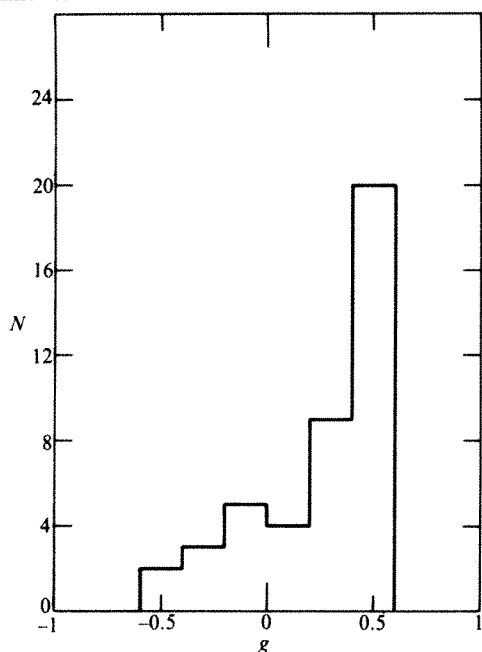


Fig. 6 Histogram showing the changes in eccentricities and semimajor axes produced by encounter with a ring-satellite in an eccentric orbit ($e_s = 0.00780$). The initial phases of the particles with respect to the satellite were chosen randomly. The strong positive bias in the distribution of $g = (e_0 - e_1)/(a_0 - a_1)$, where the subscripts 0 and 1 refer to values before and after encounter) could account for the observed eccentricity gradient ($= 0.62$) and hence the variation in width of the uranian ϵ ring.

inside and the outside of the ring, their average precession rate equals that of the ring-satellite and differential precession associated with the oblateness of the planet does not result in ring spreading. The pericentres of the ring particles remain aligned.

Stability

Even in the absence of drag, the particle path shown in Fig. 5 is not closed, since after every second encounter of a particle and the ring-satellite $|\Delta a|$ has changed in total by some small amount $\sim \pm m^n$ (see equation (3)). This suggests that the evolution of $|\Delta a|$ can be described by a random walk process and, since $n \leq 1$, that a particle will be lost from the ring after $\leq (1/2 W/m)^2$ encounters. The time between encounters is $2/3 \Delta a^{-1}$. From equation (4) we have

$$1/2 W \approx m^{1/3} \quad (11)$$

Hence, the lifetime of a ring particle is

$$\Gamma \approx \frac{T}{m^{5/3}} \quad (12)$$

where T is the orbital period of the ring satellite in arbitrary units.

Paradoxically, Γ increases as the mass of the ring satellite decreases: very narrow rings are particularly stable. For the Jupiter-Sun system $m \sim 10^{-3}$ and $T \approx 12$ yr; hence $\Gamma \sim 10^6$ yr and the lifetime of a ring orbiting the Sun with Jupiter would be short. It is probably significant that none of the Trojan asteroids are observed to have horseshoe paths. For a planetary ring, $T \sim 10^{-3}$ yr and if $m < 10^{-9}$, and this is greater than the masses needed to account for the widths of the uranian rings, then $\Gamma > 10^{12}$ yr.

Γ is not simply the lifetime of the ring, it must also be the time needed by a planet or satellite to sweep up small particles from its own orbit. If $T \sim 10^{-3}$ yr and $m < 2 \cdot 10^{-8}$, then $\Gamma > 5 \times 10^9$ yr. This suggests that very small satellites in the Solar System, which have not been captured and which lie outside the Roche zone, may be associated with narrow rings of primordial material that they have yet to accrete. We suggest that this could account for the newly discovered rings of Saturn. A ring of width ≤ 500 km

and radius 140,000 km would need only a satellite of mass $\leq 6 \times 10^{-9}$ (and diameter ≤ 200 km) to stabilise it. Such a ring would have a lifetime $\Gamma \geq 5 \times 10^{10}$ yr.

Discussion

The location of narrow rings and satellites just inside the Mimas first-order resonances supports the suggestion of Goldreich and Tremaine^{7,13} that these locations could be sites of preferential satellite formation. The observations are consistent with their theory that Mimas excited trailing spiral density waves in a disk of particles surrounding the planet at the positions of the $(p+1):p$ resonances (p is an integer) and that this resulted in the transfer of material towards the planet. Inside the Roche zone, this transfer of material has probably produced Cassini's and other divisions, the inner edges of which are associated with first-order resonances^{7,13}. We suggest here that outside the Roche zone this mechanism has resulted in satellite formation and that these small satellites maintain rings of particles they have yet to accrete.

Van Allen *et al.*⁵ claim to have detected possibly two satellites in the F ring and several small satellites outside this ring. Yet the probability of detecting a single, small satellite by their method is as low as $\sim 1\%$. In the 'ring' which they initially named the G-ring (2.490 to 2.550 R_s), both of these supposed satellites have diameters ≥ 170 km, but the separation of their orbits is only ~ 720 km (ref. 5). We suggest that their results could equally be accounted for by an eccentric ring of particles of width ≥ 170 km which is maintained by a single satellite of diameter ~ 45 km moving in an orbit of eccentricity ~ 0.005 . Similarly, the structure observed in the F-ring profile, which could be due to two satellites whose orbits are separated by only 420 km, could equally be accounted for by a ring of particles maintained by a single satellite moving in an orbit of eccentricity ~ 0.003 . Alternatively, the F and G rings could consist of a number of discrete narrow rings maintained by an equal number of small satellites. Both of these suggestions are less improbable than the suggestion of Van Allen *et al.*⁵ that on five separate occasions the Pioneer 11 spacecraft passed within a few degrees of the longitude of a small satellite.

Finally, if small satellites do exist inside the outer edges of the F and G rings, then the fact that the present orbit of Mimas is associated with the outer edges of these rings could place severe limits on changes in Mimas' orbit since the time of satellite formation. This conclusion could have profound implications with respect to the origin of resonances and the formation of satellites in the Solar System and the long-term stability of satellite orbits.

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Note added in proof: This article was written before the publication of the Pioneer 11 results in *Science*, 25 January 1980, and at the time we were not aware of the results of J. A. Simpson *et al.*¹⁴. They report that the distribution of matter in the F-ring is markedly non-uniform: the electron absorption features exhibit large radial and azimuthal variations. This supports our suggestion that the F-ring contains a number of small satellites, moving in elliptical orbits, which maintain narrow rings of particles of non-uniform width. They also report the detection of material distant 3.07 R_s from Saturn and close to the orbit of Mimas. But since the spacecraft was actually 0.04 R_s outside the orbit of Mimas at the time of the observation, we conclude that this material cannot be part of a narrow ring of the type we have described. However, since Mimas was $\sim 50^\circ$ ahead of the spacecraft at the time, the observation may be accounted for if Mimas, like Jupiter, has a number of small bodies moving in tadpole orbits about its equilateral lagrangian equilibrium points. These bodies must also be in near-resonance with Tethys, hence their orbits probably have appreciable forced eccentricities.

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Origin of rare gases in the Earth

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Although terrestrial rare gases have elemental abundances similar to those of meteorites (planetary-type rare gases), their Ne and Xe isotopic compositions are quite different which suggests that degassing of meteoritic materials alone cannot explain the atmospheric rare gases. The terrestrial rare gases must contain heavily fractionated rare gases captured by planetesimals during a fragmentation process (planetesimal-type rare gases) as well as the planetary type rare gases.

THE approximate similarity in elemental abundance pattern between the Earth and meteorites has led many investigators to assume^{1,2} that terrestrial rare gases were derived from those trapped by chondritic materials, that is, the planetary-type rare gases. However, the isotopic compositions of the atmospheric Xe and Ne are significantly different from those in the chondrites³. It is clearly not possible to derive the terrestrial Xe and Ne simply from the chondrite rare gases and the explanation for the difference should be sought in the pre-planet conditions rather than in the particular evolution processes in the Earth or in the chondrites after their formation³. Astrophysical considerations also indicate that the grains condensed from the solar nebula which subsequently formed the Earth had mostly existed around the present Earth orbit⁴, whereas chondrite parent bodies are generally regarded to have formed further from the Sun, most likely near the asteroidal belt (~2–4 AU) (refs 5, 6). Because the physical conditions in the solar nebula such as temperature and pressure must be different between the asteroidal belt and the Earth orbit, there seems no compelling reason to suppose that

the original Earth materials had trapped the rare gases in the same way as did the chondritic meteorites or their parent bodies. For example, the low temperature adsorption hypothesis⁷ which was proposed to explain the chondritic rare gas pattern is not applicable to the Earth, as the nebula temperature around 1 AU was too high, ~225 K (ref. 8) for grains to adsorb any significant amount of rare gases. This consideration does not, however, rule out the possibility that some of the terrestrial rare gases were supplied from the chondrites, as part of the chondritic materials must have been brought to the Earth's region by the gas drag force and mixed with the materials which formed the Earth⁹. In fact, chondritic materials must be called for to explain the material balance, particularly that of the volatile elements in the Earth^{10,11}. The past decade has seen considerable progress in astrophysics, such as the discovery of the fragmentation process^{12–14} and the subsequent formation of planetesimals. We show here that astrophysical theory leads to a conclusion that a substantial amount of gravitationally-fractionated rare gases must have been captured by planetesimal which later formed the Earth. Hence, the terrestrial rare gases consist of both these planetesimal type rare gases and those trapped in the chondritic materials, that is, the planetary type rare gases. This mixing model can explain the large isotopic fractionation of the terrestrial Xe relative to the planetary and solar type Xe. Except for the Kr and Ar ratio, the model is also consistent with other characteristics of the terrestrial rare gases.

Constraints on terrestrial rare gases

The characteristic features of the rare gases in the Earth which any theory should explain are summarised below. (For the detailed compilation of the terrestrial rare gas characteristics outlined here, see ref. 3.)

(1) The rare gases are depleted relative to the solar abundance. The depletion ranges from 10^{-6} (Xe) to 10^{-11} (Ne). The elemental abundance pattern is similar to that of the planetary type rare gases except for Xe, the latter being more depleted in the Earth.

(2) The isotopic composition of terrestrial Xe shows large mass-dependent fractionation relative to the planetary Xe, in that the heavier isotopes are more enriched (about 4% per AMU). The pattern is surprisingly linear with mass number for all but ^{129}Xe and the heaviest isotopes (see Fig. 2). Departure from the linear fractionation at the heavy end is usually attributed to fission effects, and at ^{129}Xe to extinct radioactive ^{129}I .

(3) Ne isotopic abundance is different both from the solar and planetary Ne (Ne B and Ne A respectively), in that the terrestrial

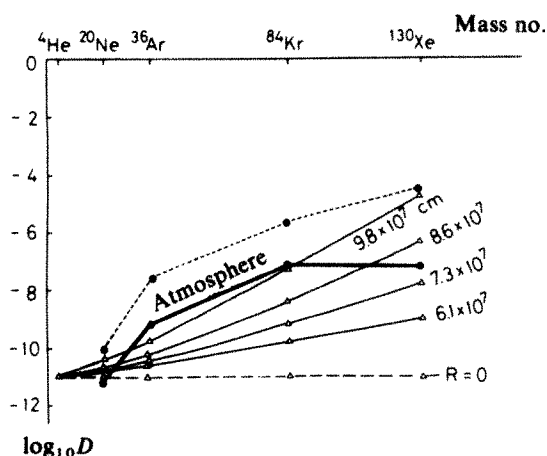


Fig. 1 Rare gas elemental abundance ($\log_{10} D$) relative to the solar abundance in planetesimals of various sizes (R) are plotted against a mass number. The amount of rare gases is normalised to Si. In the case of the atmospheric values (thick line), M_i stands for the rare gases in the atmosphere and Si in the whole Earth. Note that the mixing of the gravitationally fractionated planetesimal type (thin lines) and the planetary type rare gases (dotted line) would approximate the atmospheric pattern. [$D = (M_i/\text{Si})_{\text{atm}} / (M_i/\text{Si})_{\text{solar}}$].

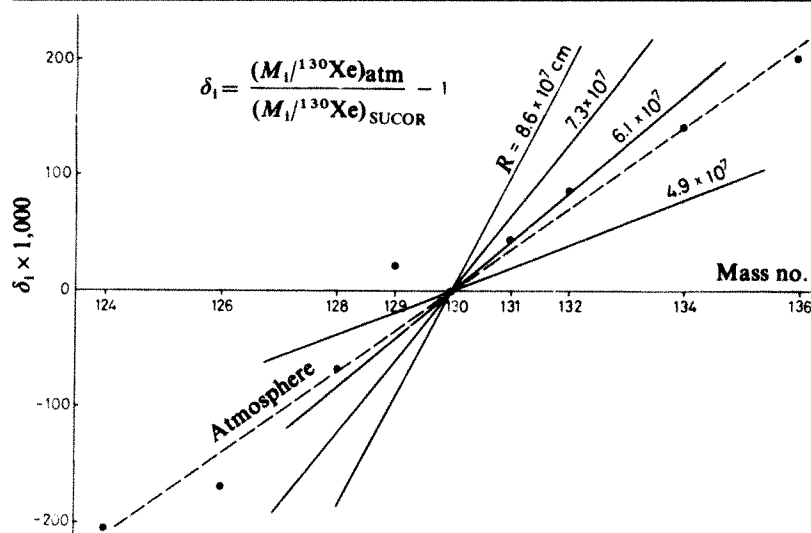


Fig. 2 Isotopic fractionation pattern for Xe in planetesimals of various sizes relative to SUCOR Xe (ref. 3) (SUCOR designates surface-correlated gas in lunar soil, which is regarded to be close to the solar composition). Lines indicate the fractionation for a planetesimal with a radius R (cm), which resulted from the gravitational fractionation in the planetesimal.

Ne lies roughly midway between the two types in a $^{20}\text{Ne}/^{22}\text{Ne}$ - $^{21}\text{Ne}/^{22}\text{Ne}$ diagram.

(4) In spite of the large difference in Ne and Xe isotopic compositions between the Earth and chondrites, Ar, and Kr seem to exhibit nearly the same isotopic compositions, although terrestrial Kr shows slight enrichment in the lighter isotopes of $\sim 0.5\%$ per AMU. However, the constraints (3) and (4) may not be as stringent as the constraints (1) and (2), because of uncertainties in defining the representative planetary Ne and Kr isotopic ratios.

Planetesimal type rare gases

There are numerous astrophysical theories of the origin of the Solar System and the formation of the planets. Here, we follow the astrophysical theories developed by Hayashi and his colleagues^{13,15-17}. According to these authors, grains condensing from the solar nebula gradually sedimented to the median plane. When the sedimented grain density exceeds a critical value ($\sim 10^{-7} \text{ g cm}^{-3}$), the grains would suddenly collapse to form numerous planetesimals, of size about 10^{18} g . This process is called fragmentation of the dusty layer and was obtained from the astrophysical dynamics independently by Safronov¹², Hayashi¹³ and Goldreich and Ward¹⁴. As the fragmentation occurred $\sim 10^4$ - 10^5 yr after the formation of the proto-Sun¹⁶, there still existed solar nebula gas during the fragmentation. The planetesimals thus formed were loosely-compacted grain ensembles, in that the central pressures were ~ 1 - 10^2 atm for planetesimals with radii 10^6 - 10^7 cm respectively¹⁷. Hence, there must have been considerable inter-grain free space or porosity, which was filled with the solar nebula. The time constant for the heavier rare gases to reach gravitational equilibrium within a planetesimal of a radius R (cm) is of the order of $R^2/(4 \times 10^{10}) \text{ yr}$ (R in cm), whereas the growth time for a planetesimal is $\sim 10^4$ yr (ref. 18). Hence, the rare gases in a planetesimal with a radius less than a few hundred kilometres are in gravitational diffusion equilibrium. When a planetesimal grows further, the gases are no longer in equilibrium and are 'frozen' in the last equilibrium state, corresponding to a planetesimal with a radius of a few hundred kilometres. The rare gases thus frozen in the planetesimals were finally incorporated in the terrestrial planets.

For rare gases in gravitational equilibrium within the planetesimals the density distribution can be expressed as

$$\frac{1}{\rho_i} \frac{dP_i(r)}{dr} = -\frac{GM(r)}{r^2} \quad (1)$$

$$M(r) = \frac{4}{3} \pi r^3 \rho_s$$

where ρ_i , ρ_s are the densities of the rare gas i and the planetesimal solids (assumed to be constant), P_i the pressure of the rare

gas i , G the gravitational constant, M the mass of the planetesimal, r the distance from the centre of the planetesimal. Assuming that the rare gases follow the equation of state for an ideal gas and that the temperature is constant, equation (1) yields:

$$\rho_i(r) = \rho_i(R) \exp(A\mu_i(1-x^2))$$

$$A = \frac{H}{kT} \frac{2\pi}{3} \rho_s GR^2$$

$$x = r/R$$

where k is Boltzmann's constant, T the absolute temperature, μ_i the atomic weight of the rare gas i , H the mass of hydrogen atom, R the radius of the planetesimal and $\rho_i(R)$ is assumed to be equal to the density in the surrounding nebula at the surface of the planetesimal ($r = R$). Hence, assuming a constant porosity s for various size of planetesimals, the total mass M_i of the rare gas in the planetesimal is written as,

$$M_i = 4\pi s \int_0^R r^2 \rho_i dr \quad (2)$$

$$= 4\pi R^3 s \rho_i(R) \exp(A\mu_i) \int_0^1 x^2 \exp(-A\mu_i x^2) dx$$

We then define a retention factor iD

$${}^iD = (M_i/M_{\text{Si}})_p / (M_i/M_{\text{Si}})_{\text{solar}}$$

where Si denotes a conventionally chosen standard element Si and the subscript p refers to a planetesimal. Considering that the mass ratio of the solid phase to the gas phase in the condensing solar nebula is 0.00343 (ref. 19), the retention factor iD can be expressed with the aid of equation (2),

$${}^iD = 0.00343 \times s \rho_g / \rho_s$$

$$\times 3 \exp(A\mu_i) \int_0^1 x^2 \exp(-A\mu_i x^2) dx \quad (3)$$

or

$${}^iD = ({}^iD)_0 \alpha_i$$

$$({}^iD)_0 = 0.00343 \times s \rho_g / \rho_s$$

$$\alpha_i = 3 \exp(A\mu_i) \int_0^1 x^2 \exp(-A\mu_i x^2) dx$$

where $({}^iD)_0$ corresponds to the retention factor for the planetesimal with a negligible gravitation ($A \rightarrow 0$), ρ_g nebula gas pressure and α_i indicates the enrichment factor of the rare gas i due to the gravitational force of the planetesimal.

In Table 1, we show the values of α_i for planetesimals of various sizes. To calculate α_i , we took $\rho_s = 2$ (in cgs) and $T = 225 \text{ K}$, the values being estimated for the solar nebula at 1 AU

Table 1 Enrichment factor α_i for rare gas i in planetesimals with various radius R

$R(\times 10^7 \text{ cm})$	α_i				
	^4He	^{20}Ne	^{36}Ar	^{84}Kr	^{130}Xe
0.12	1.00	1.00	1.00	1.00	1.00
1.2	1.00	1.02	1.03	1.08	1.13
6.1	1.10	1.64	2.61	14.3	99.5
7.3	1.18	2.10	4.34	67.3	1.46×10^3
8.6	1.20	2.87	8.48	500	4.1×10^4
12	1.47	12.2	190	2.9×10^6	8.7×10^{10}

(ref. 8). For the smaller planetesimals ($R \leq 10^6 \text{ cm}$) the enrichment is negligible, whereas a planetesimal with $R = 8 \times 10^7 \text{ cm}$, Xe enrichment amounts to more than 10^4 .

Figure 1 displays the relative elemental abundances of the planetesimal type rare gases normalised to the solar abundances, for which we used a value of $\rho_s = 3 \times 10^{-8}$ (in cgs) at 1 AU (ref. 8) and $S = 0.33$ deduced as the ratio of $(\rho - \rho_s)$ to the density of crystalline silicate ($\rho \approx 3$). The horizontal line (dashed line) represents the case $A = 0$ (no gravitational field), for which $\sim 10^{-11}$ depletion occurs in all rare gases, corresponding to the ratio of the solid to the gas phases in the planetesimal. Figure 1 also shows the rare gas abundance patterns in both the terrestrial atmosphere (thick line) and the planetary type rare gases (dotted line) relative to the solar abundance. Although the planetesimal type rare gases can roughly account for the elemental abundance, the pattern is not exactly the same as that observed in the atmosphere. As discussed above, we must also take into account the planetary type rare gases which were introduced by chondritic materials in the terrestrial atmosphere. A mixing of the two types would better approximate the atmospheric rare gas abundance pattern.

Rare gas isotopic fractionations in planetesimals

The gravitational mass fractionation mechanism also leads to an isotopic fractionation. Isotopic fractionation is conveniently expressed in terms of δ -values relative to the solar isotopic composition. In the case of Xe δ_i will be defined conventionally as

$$\delta_i = (i\text{Xe}/^{130}\text{Xe})/(i\text{Xe}/^{130}\text{Xe})_{\text{solar}} - 1$$

δ_i is then expressed in terms of the retention factor iD , and thus of α_i defined in equation (3),

$$\delta_i = (D_i)/(D^{130}\text{Xe}) - 1 = (\alpha_i)/(\alpha^{130}\text{Xe}) - 1 \quad (4)$$

As $\mu_i \gg 1$ and $\mu_i \gg \Delta_i \equiv |\mu_i - 130|$ for the Xe isotopes, equation (4) can be approximately expressed as

$$\delta_i \approx \left\{ A - \frac{3}{2} \frac{1}{130} \left(1 - \frac{1}{\alpha_i} \right) \right\} \Delta_i$$

Hence, the gravitational mass fractionation indicates a linear mass fractionation trend in agreement with the observation. Table 2 shows the isotopic fractionation δ_i per atomic mass unit for various A , that is, for the planetesimals with various radius R . The isotopic mass fractionation is larger for the heavier rare gases and for the larger planetesimals.

Table 2 Isotopic fractionation factor δ_i (see text for the definition) for rare gas i in planetesimals with various radius R

$R(\times 10^7 \text{ cm})$	δ_i			
	^{20}Ne	^{36}Ar	^{84}Kr	^{130}Xe
1.2	$< 10^{-4}$	$< 10^{-3}$	$< 10^{-3}$	$< 10^{-3}$
6.1	$< 10^{-2}$	0.01	0.02	0.03
7.3	0.01	0.02	0.03	0.04
8.6	0.02	0.04	0.06	0.07
12	0.16	0.18	0.20	0.21

For planetesimals larger than $8 \times 10^7 \text{ cm}$, Xe isotopic fractionation amounts to $\sim 7\%$ per AMU. The δ_i values or a mass fractionation line calculated for various size of planetesimals are shown in Fig. 2 together with the case for the atmospheric Xe. Figure 2 shows that the atmospheric Xe mass fractionation trend can be explained as a mixture of fractionated planetesimal type Xe with the planetary type Xe which shows little mass fractionation relative to the solar isotopic ratio. Owing to the small mass number, the planetesimal Ne isotope shows little mass fractionation relative to the solar type Ne. Hence, we expect from the mixing model that the terrestrial Ne has the isotopic composition somewhere between the planetary and the solar type rare gases; this is, in fact observed in the terrestrial Ne (ref. 3). Because of the particular shapes of the retention factor D for the planetesimal and the chondrite (Fig. 1), that is, upward concave for the former and downward concave for the latter, the planetesimal type Ar and Kr could be less conspicuous in a mixture in which planetesimal gas is the major contributor of Ne and Xe. This may account for the approximate similarity in the Ar and Kr between the planetesimal and planetary-type rare gases, while Xe and Ne isotopic compositions are significantly different from each other.

Origin of the rare gases in the Earth

We first adopt the generally accepted assumption that the terrestrial atmosphere is of a secondary origin, in that the atmosphere was degassed from the interior of the Earth²⁰. We also assume that the atmospheric rare gases essentially represent the rare gases in the whole Earth.

We conclude that the terrestrial rare gases can be interpreted as a mixture of planetary type rare gases which were added to the Earth by chondritic materials and the planetesimal type rare gases captured by planetesimals which later accreted the Earth. As we do not know the exact size distribution of the planetesimals which later accreted the Earth, it would be meaningless to attempt a quantitative estimation of the degree of mixing between the two types of the rare gases. Here we would only suggest that mixing of roughly equal amounts of the planetary- and planetesimal-type rare gases can explain some of the observed characteristic of the terrestrial rare gases: (1) the enormous depletion of the rare gases relative to the solar abundance, with the light rare gases more depleted; (2) the large Xe isotopic fractionation ($\sim 4\%$ per AMU) with nearly a linear mass dependence; (3) the difference in Ne isotopic compositions among terrestrial, planetary, and solar type rare gases. The situation here—previously seen as a mixing of the two latter to make the first—has not been affected by our model because the planetesimal modifications of the isotopic composition of neon should be small. On the other hand, difficulties for the model are the isotopic uniformities everywhere for argon and krypton because we would expect the planetesimal effects on argon, and especially krypton, to introduce effects which are not, in fact seen. Also, the model does not offer a direct explanation of the Xe deficiency in the atmosphere relative to the planetary type rare gases. We suggest that the deficiency may be due to the difficulty in its degassing from the Earth.

For the origin of the planetary type rare gases, we offer no specific explanation; it could be low temperature adsorption⁷, or crystal growth trapping²¹ or others²²⁻²⁴, but the specific mechanism for the origin is not important in the present discussion. Finally, the mixture model should also apply to other inner planets. We therefore suggest that Xe and Ne isotopes should show similar isotopic fractionation trends relative to the solar type isotopic compositions to those observed in the terrestrial rare gases.

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Synthesis in *E. coli* of a polypeptide with human leukocyte interferon activity

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Double-stranded cDNA prepared from the 12S fraction of poly(A) RNA from interferon (IF)-producing human leukocytes was cloned in Escherichia coli using the pBR322 vector. One of the resulting clones had a 910-base pair insert which could hybridise to IF mRNA and was responsible for the production of a polypeptide with biological IF activity. Up to 10,000 units IF activity per g of cells was obtained from some clones.

CELLS of almost all vertebrates, when exposed to certain viruses or inducers, produce one or more (glyco)proteins, known as interferons^{1,2}. Interferons (IFs) are characterised biologically by their ability to induce in target cells a virus-resistant state which is associated with the *de novo* synthesis of several proteins, in particular a protein kinase³, an oligoisoadenylate synthetase^{4,5} and a phosphodiesterase⁶. In addition, IFs have a regulatory effect on the immune response^{7,8} and their enhancement of

killer lymphocyte activity⁹ may be the basis of their inhibitory effect on tumour growth².

Two major classes of acid-stable (type I) IFs have been recognised in man—leukocyte interferon (Le-IF), released by stimulated leukocytes, and fibroblast interferon (F-IF), produced by stimulated fibroblasts. Le-IF and F-IF differ not only immunologically but also in their target cell specificity: whereas both IFs induce a virus-resistant state in human cells, Le-IF is also very active on bovine, porcine and feline cells, whereas F-IF is not². The two IFs are encoded by separate mRNAs¹⁰.

Human Le-IF has been purified more than 80,000-fold, to a specific activity of 4×10^8 units per mg (ref. 11) or 2.5×10^8 units per mg (ref. 12). Two components have been characterised by polyacrylamide gel electrophoresis, with apparent molecular weights (MWs) of 21–22,000 and 15–18,000, respectively^{13,14}; they are believed to differ in their degree of glycosylation¹⁴. Enzymatic¹⁵ or chemical¹⁶ removal of most or all of the carbohydrate moiety seems to have little effect on the biological activity of IF.

We sought to clone human Le-IF cDNA in order to construct bacterial strains producing polypeptide(s) with human IF activity, and to generate the tools required for the analysis of Le-IF gene structure and function. The particular difficulties of this undertaking were the lack of a purified Le-IF mRNA and our ignorance of the structure of Le-IF, which precluded the preparation of pure or highly enriched IF cDNA, or of a probe for the identification of the desired clones.

We describe here the isolation of a hybrid plasmid containing a 872-base pair Le-IF cDNA, which elicits the formation in *Escherichia coli*, of a polypeptide with the immunological and biological properties of human Le-IF.

Isolation of hybrid plasmids containing IF cDNA sequences

Hybrid DNA, consisting of leukocyte cDNA sequences joined to pBR322 at the *Pst*I site by means of dG:dC sequences, was prepared by conventional means, using as starting material a 12S fraction of poly(A) RNA from IF-producing leukocytes, purified about 10-fold for IF mRNA. cDNA cloned in this fashion is usually flanked by *Pst*I sites and, as it is located in the

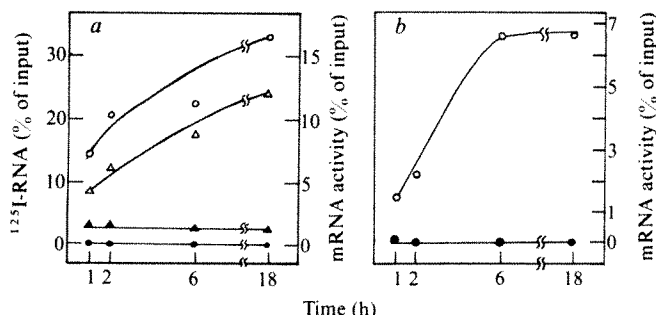


Fig. 1 Hybridisation of IF mRNA and ¹²⁵I-globin mRNA to filter-bound DNAs. DNA was linked to DPT paper as described elsewhere³⁶. *a*, For each time point, one 0.25-cm² piece of DPT paper with 500 ng of *Hind*III-excised insert of rabbit β-globin cDNA plasmid and one piece with 700 ng of *Hind*III-digested pBR322 were hybridised as a sandwich with 10 μl of hybridisation medium²² containing 200 ng ¹²⁵I-labelled globin mRNA. *b*, As above except that DPT papers contained 250 ng of *Hind*III-excised insert of rabbit β-globin cDNA plasmid and 250 ng of *Pst*I-excised insert of Hif-2h, respectively, and hybridisation was with 5 μg of Le poly(A) RNA in 10 μl. In all cases hybridisation, washing and elution were as described in ref. 22. The RNA was recovered, its ¹²⁵I radioactivity determined, and injected into 40–50 oocytes. In experiment *b*, oocytes were incubated with 50 μCi ³H-histidine. Oocyte supernatants were assayed for IF activity by the cytopathic effect reduction assay (see Table 3 legend). Le poly(A) RNA (1 μg) injected directly into 20 oocytes gave 2,700 units IF. For the determination of ³H-labelled globin formation, oocytes were homogenised, centrifuged and an aliquot of the supernatant electrophoresed through a 20% polyacrylamide gel³⁷. The globin band was cut out and the radioactivity determined in toluene-based scintillator solution. 100 ng globin mRNA injected directly gave 100,000 c.p.m. ³H-globin. *a*, ¹²⁵I-RNA hybridised to β-globin cDNA (○) or to pBR322 (●). ³H-labelled β-globin formed in oocytes after injection of RNA hybridised to β-globin cDNA or (Δ) pBR322 (▲). *b*, IF activity formed in oocytes after injection of RNA hybridised to Hif-2h fragment (○) or β-globin cDNA (●).

Table 1 mRNA hybridisation translation assay for the detection of IF cDNA in hybrid DNA from pools of transformed *E. coli*

DNA sample	Interferon activity
Expt 1: Pools of 512 clones	
I	<60 (<60); <u>110</u> (<20); <110 (<110); <110 (<110); <35 (<35)
δ	<u>20</u> (<20)
N	<u>35</u> (<20); <110 (<110); <u>200</u> (<110)
A	<60 (<60); <u>60</u> (<20); <110 (<110); <110 (<110)
8 other groups negative	
Expt 2: Pools of 64 clones from sample A	
A-I	<35 (<35); <35 (<35)
A-II	<u>130</u> (<30); <45 (<45)
A-III	<u>225</u> (<35); <u>35</u> (<30); <u>35</u> (<30); <u>600</u> (<30); <20 (<20)
A-IV	<u>85</u> (<35); <25 (<25)
A-V to VIII	negative
Expt 3: Pools of 8 clones from sample A-III	
A-III-1	<20 (<20); <20 (60); <u>35</u> (<30)
A-III-2	<35 (<35); <30 (<30); <u>150</u> (<20); <u>600</u> (<35); <u>110</u> (60)
A-III-3	<25 (<25); <30 (<30)
A-III-4	<u>30</u> (<30); <20 (<20); <20 (60)
A-III-5 to 8	negative
Expt 4: Single clones from sample A-III-4	
A-III-4B	<35* (<35); <20 (60)
A-III-4C	<u>35</u> (60); <u>60</u> * (<35); <u>111</u> * (<11); <11 (<11); <u>20</u> (<20)

Hybrid DNA containing leukocyte cDNA was prepared as follows. To obtain poly(A) RNA from IF-producing leukocytes, 10^{11} human leukocytes were primed with Le-IF and induced with Sendai virus as described elsewhere²¹. After 5 h at 37°C the cells were collected, suspended in 1 l PBS and added to 17 l 20 mM Tris-HCl (pH 7.5), 1 mM EDTA and 2% SDS. The lysate was digested with Pronase (200 μ g ml⁻¹) for 1 h at 20°C. 2 M Tris-HCl buffer (pH 9, 5% vol) was added and the solution extracted with 15 l phenol for 30 min. Chloroform (3 l) was added to aid phase separation, the aqueous phase adjusted to 0.3 M NaOAc buffer (pH 5.5) and the nucleic acid precipitated with ethanol. The precipitate (about 1 g) was dissolved in 900 ml TNE [Tris-HCl (pH 7.5), 100 mM NaCl, 5 mM EDTA] containing 0.5% SDS, extracted three times with phenol and exhaustively with ether, and the poly(A) RNA recovered by three batch adsorptions to 3 \times 5 g oligo(dT) cellulose (type 7, P-L Biochemicals) followed by elution with water. The yield was 1.6 mg; 1 μ g gave rise to 300 units IF when injected into oocytes. For further purification, 860 μ g RNA in 5 mM EDTA were passed through a Chelex-100 column, heated for 90 s at 100°C and centrifuged through a 5–23% sucrose gradient in 50 mM Tris-HCl (pH 7.5), 1 mM EDTA and 0.2 M NaCl. The fractions containing the IF-mRNA activity, sedimenting around 12S, were pooled and the poly(A) RNA recovered by oligo(dT) cellulose chromatography. The yield was 40 μ g; 1 μ g gave rise to 3,600 units IF when injected into oocytes. pBR322-linked Le-cDNA was prepared essentially as described previously²². Sucrose-gradient purified poly(A) RNA from two preparations (48 μ g) was used as template for reverse transcriptase to generate 10 μ g cDNA 600–1,000 nucleotides long; this was converted into double-stranded DNA by DNA polymerase I, and treated with S₁ endonuclease (yield, 8 μ g preparation A). Of this DNA, 5 μ g were centrifuged through a sucrose-density gradient, and material sedimenting faster than a 600-base pair ³²P-DNA marker was pooled and precipitated with ethanol (preparation B, 3 μ g). cDNA was elongated with dCMP residues, annealed to dGMP-elongated, PstI-cleaved pBR322 (ref. 17) and used to transform *E. coli* χ 1776 (ref. 23) (preparation A; 3.3×10^4 tetracycline-resistant transformants per μ g DNA), or *E. coli* HB101 (preparation B; 4×10^4 transformants per μ g DNA). Ten thousand colonies of transformed *E. coli* χ 1776 were inoculated individually into wells of microtitre plates and stored with 20% glycerol at –20°C. Five thousand colonies of transformed *E. coli* HB101 (from preparation A) were raised on Millipore filters and stored frozen as described by Hanahan and Meselson^{22,24}. To carry out the hybridisation translation assay, the number of bacterial clones (from preparation A) indicated in the table were inoculated individually on to agar plates, incubated for 24 h and washed off with medium. This suspension was used to inoculate 1–1 cultures from which plasmid DNA was purified as described (method B in ref. 25). The hybrid Le cDNA (20 μ g) was cleaved with HindIII, mixed with 12 μ g Le poly(A) RNA, 5 ng ¹²⁵I-labelled rabbit β -globin mRNA (5,000 c.p.m.) and 0.1 μ g PstI-cleaved rabbit globin cDNA plasmid (Z-pBR322(H3)/Rc β G-4.13)²⁶ in 40 μ l 80% formamide, 0.4 M NaCl, 10 mM PIPES buffer (pH 6.4) and 5 mM EDTA, and heated for 4 h at 56°C. After diluting to 1 ml with 0.9 M NaCl and 0.09 M trisodium citrate (pH 7) and adjusting to 4% formamide, the solution was filtered through a Millipore filter (13 mm diameter, 0.4 μ m pore size). The filter was washed for 10 min at 37°C in 0.15 M NaCl, 0.015 M trisodium citrate and 0.5% SDS, and the RNA recovered by heating the filter for 5 min in 0.5 ml 1 mM EDTA, 0.5% SDS and 3 μ g ml⁻¹ yeast RNA at 75°C. The RNA was purified by oligo(dT) cellulose chromatography, precipitated with ethanol and assayed for IF-mRNA activity. To determine IF-mRNA activity, the RNA sample (up to 3 μ g) was dissolved in 1–3 μ l 15 mM Tris-HCl (pH 7.5) and 88 mM NaCl, and injected into 20–60 *Xenopus laevis* oocytes²⁷ (50 nl per oocyte). Oocytes were incubated for 12–16 h in Barth's medium²⁸, homogenised in 0.5 ml 50 mM Tris-glycine buffer (pH 8.9) and the supernatant was assayed for IF. In later experiments (Table 2), incubation was for 24–48 h and the incubation medium was assayed for excreted IF²⁹. IF was determined by the vesicular stomatitis virus (VSV) plaque reduction assay²¹. All values are expressed in international units. Values marked with an asterisk were obtained by hybridisation to diazobenzoyloxymethyl (DBM)-bound DNA, as described in ref. 22. Underlined values are considered positive. The control values (in parentheses) were obtained by hybridisation to pBR322 DNA. All manipulations involving live *E. coli* HB101 or *E. coli* χ 1776 containing Le cDNA-pBR322 hybrids were carried out in P3 containment conditions as described in the NIH Recombinant DNA Research Guidelines.

β -lactamase gene, can be expressed as a fused protein¹⁷ or, in certain circumstances, as an independent polypeptide¹⁸.

We identified an IF cDNA clone by a mRNA hybridisation translation assay¹⁹. Hybrid plasmid was prepared from pools of 512 bacterial clones, and 20 μ g of each plasmid pool were cleaved with PstI, denatured and annealed with 12 μ g crude Le poly(A) RNA. ¹²⁵I-labelled globin mRNA and rabbit β -globin cDNA plasmid were added to monitor hybridisation and all subsequent steps. Hybridised RNA was recovered from the filters, purified and injected into oocytes to determine its IF-mRNA activity. Control hybridisations were carried out with pBR322. The overall recovery of β -globin mRNA activity was only about 5% of the input.

Four out of 12 groups of 512 clones (δ , λ , I and N) gave positive results by this assay, albeit erratically; controls were consistently negative in these groups (Table 1). However, in later experiments, controls occasionally gave a positive result, perhaps due to insufficient washing of the filters. A group of clones was scored as being positive if the value was higher than in the parallel control. The bacterial clones of group λ were arranged in 8 subgroups of 64 each, and assayed as above. Three of these subgroups, λ -II, λ -III and λ -IV, gave positive responses; the clones of λ -III were regrouped into eight sets of eight.

The set λ -III-4 was the first to yield a positive result; λ -III-2 subsequently also gave positive results. DNA was prepared from the single λ -III-4 clones and that from λ -III-4C gave positive responses both by liquid and filter-bound hybridisation (Table 1). After recloning in *E. coli* HB101 the hybrid plasmid of clone λ -III-4C, designated Z-pBR322(Pst)/HcIF-4c (abbreviated to Hif-4c), was purified and cleaved with PstI; it released a 320-base pair insert, that is, a fragment about one-third of the expected length of complete IF cDNA. The fragment bound IF mRNA efficiently (Table 2).

A set of colonies containing hybrid DNAs related to Hif-4c was identified by *in situ* hybridisation with ³²P-labelled Hif-4c PstI fragment. Among the 64 clones of λ -III, three gave a strong hybridisation response, namely 4C, 2H and 7D, and two (1E and 3D) a weak one. λ -III-2H had the largest insert, about 900 base pairs; it was recloned in *E. coli* HB101 and designated Z-pBR322(Pst)/HcIF-2h (abbreviated to Hif-2h). In addition, 5,000 clones prepared as described, but using double-stranded Le-IF cDNA selected for length above 600 base pairs (preparation B, cloned in *E. coli* HB101), were screened by *in situ* hybridisation, using the same probe. Of 185 positive clones identified, 95 gave a strong and 90 a weak hybridisation response in the Grunstein-Hogness assay²⁰. The former were

designated *E. coli* HB101(Z-pBR322(*Pst*)/HcIF-SN1 to -SN95) (abbreviated to SN1 to SN95).

Properties of plasmid Hif-2h

The insert of plasmid Hif-2h, released by *Pst*I cleavage, was attached to diazophenylthioether (DPT) paper and the kinetics of hybridisation to IF mRNA in conditions of DNA excess determined (Fig. 1). In optimal conditions, about 7% of the IF-mRNA activity and 12% of the β -globin-mRNA activity were recovered, as measured in the oocyte system. Thus, the insert of Hif-2h hybridises to IF mRNA with about the same efficiency as does β -globin cDNA to β -globin mRNA.

Restriction and sequence analysis of Hif-2h (M. Schwarzstein, N. Mantei and M.S., unpublished results) showed that the insert has 910 base pairs of which 23 are 5'-terminal and 15 are 3'-terminal GC pairs; there is one site each for *Bsp*I (85), *Bgl*II (335) and *Eco*RI (710) endonucleases, two sites for *Pvu*II (125, 425), and three sites for *Ava*II (190, 385, 655) and none for *Hha*I, *Taq*I, *Hind*III, *Hpa*II, *Pst*I and *Bam*HI. (The values in parentheses indicate the distance in base pairs from the *Pst* terminus corresponding to the 5' end of the mRNA.) The orientation of the cDNA insert, as ascertained by nucleotide sequence analysis, was such that the reading direction of the IF cDNA coincided with that of the β -lactamase gene.

Detection of IF activity in *E. coli* strains transformed with Hif-4c-related hybrid plasmids

The isolated Hif-2h *Pst*I fragment was joined to *Pst*I-cleaved pBR322 and to three plasmids, pKT279, pKT280 and pKT287, derived from pBR322 by deletions in the β -lactamase gene; DNA ligated into the *Pst* site of this set can be translated in the three possible reading frames by readthrough from the β -lactamase sequence (K. Talmadge, personal communication). *E. coli* HB101 strains transformed with these hybrid DNAs were *E. coli* HB101 (Z-pBR322(*Pst*)/HcIF-2h-AH1 to -AH4), *E. coli* HB101 (Z-pKT279(*Pst*)/HcIF-2h-AH1 to -AH8) and so forth, or in abbreviated form, 322-AH1 to -AH4, 279-AH1 to -AH8, and so on. S-30 or S-100 extracts, from 24 of the AH and 49 of the SN strains grown to stationary phase, were tested for IF activity. The original Hif-2h-containing strain and many of the AH strains showed IF activity; three of them, 279-AH8, 280-AH3 and 287-AH6, were selected for further testing. Of

Table 2 Characterisation of the insert of hybrid plasmid Hif-4c by the mRNA hybridisation translation assay

DNA fragment	Amount of leukocyte poly (A) RNA (μ g)	Time of hybridisation (h)	IF activity (units ml ⁻¹)
Hif-4c	2.5	16	250; 100
β -globin cDNA	2.5	16	4; 1
Hif-4c	7.5	16	3,000; 1,000
β -globin cDNA	7.5	16	4; 30
Hif-4c	7.5	5	1,000; 1,000
β -globin cDNA	7.5	5	10; 1

The insert of plasmid Hif-4c was excised with *Pst*I, purified by electrophoresis through a 2% agarose gel and recovered by successive adsorption to and elution from hydroxyapatite and DEAE cellulose. 120-ng fragments were linked to each 0.25 cm² DPT paper (B. Seed, personal communication). Pre-hybridisation, hybridisation and elution of RNA were as described elsewhere²². The RNA was injected into oocytes and IF activity was determined after 48 h by the cytopathic effect reduction assay³⁰ (see Table 3 legend).

the 49 SN strains, 16 had IF activity; two of the highest producers, SN35 and SN42, and a negative control, SN32, were further examined. Table 3 shows the results obtained with S-100 extracts of log phase bacteria. IF activities ranged from 100 to 1,000 units per ml of S-100 extract derived from a 20-ml resuspension of the 2.0 g (approximately) of bacterial cells contained in 1 l of culture.

Characterisation of the IF activity produced in transformed *E. coli*

We tested the sensitivity of the IF activity to a protease by incubating S-100 extracts of 287-AH6 and SN35 for 30 min at 37°C with increasing amounts of trypsin. As a control, authentic human Le-IF was mixed with the (inactive) S-100 extract of SN32 (to give a similar protein concentration, 6 mg ml⁻¹) and digested in parallel. In all cases, the activity was partially abolished at 200 μ g ml⁻¹ and completely abolished at 1 mg ml⁻¹ trypsin.

Table 3 IF activity in extracts of transformed *E. coli*

S-100 extracts of <i>E. coli</i> HB101 transformed by:	IF activity (units per ml extract)
a Z-pBR322(<i>Pst</i>)/HcIF-2h	100; 100
b Z-pKT279(<i>Pst</i>)/HcIF-2h-AH8	100; 300
c Z-pKT280(<i>Pst</i>)/HcIF-2h-AH3	1,000; 1,000
d Z-pKT287(<i>Pst</i>)/HcIF-2h-AH6	200; 200
e Z-pBR322(<i>Pst</i>)/HcIF-SN35	1,000; 1,000
f Z-pBR322(<i>Pst</i>)/HcIF-SN42	300; 100
g Z-pBR322(<i>Pst</i>)/HcIF-SN32	0; 0

The IF-cDNA insert of Hif-4c, excised with *Pst*I and purified as described in Table 2 legend, was joined to *Pst*I-cleaved pKT279, pKT280 and pKT287, respectively. *E. coli* HB101 was transformed with these products and tetracycline-resistant colonies were screened by *in situ* hybridisation²⁰ as described by Hanahan and Meselson²⁴, using the Hif-4c *Pst*I fragment nick-translated with [α -³²P]dATP (1,100 Ci mmol⁻¹, NEN) and [α -³²P]dCTP (470 Ci mmol⁻¹, NEN) as labelled substrates³¹. Three clones (b-d) were selected in preliminary assays for IF activity. Clones c-g were from a set of IF-cDNA-containing clones identified among 5,000 *E. coli* HB101 transformed with Le cDNA (preparation B, see Table 1 legend) by *in situ* hybridisation as above. e and f were shown to produce IF in a preliminary screening, and g was chosen as negative control. One-litre cultures of transformed *E. coli* were grown to an A₆₅₀ of about 0.8. The cells (about 2.0 g) were collected, washed with 50 mM Tris-HCl (pH 8), 30 mM NaCl and resuspended in 20 ml of the same buffer. Lysozyme was added to 1 mg ml⁻¹; after 30 min at 0°C, the suspension was frozen and thawed five times and centrifuged at 10,000 r.p.m. for 20 min. The supernatant was centrifuged at 40,000 r.p.m. for 1 h in a Spinco 60 rotor. The S-100 supernatants (about 6 mg ml⁻¹ protein in all cases) were assayed in duplicate by the cytopathic effect reduction assay and their IF content estimated relative to a standard IF preparation. IF activity was determined by the cytopathic effect reduction assay as follows. The IF samples, serially diluted 1:3 were mixed with 10⁵ CCL23 cells in the wells of a microtitre plate (Cooke) in MEM-10% newborn calf serum. After 24 h the medium was replaced by an appropriate dilution of Mengo virus in the same medium. 24 h later the medium was replaced with 0.5% crystal violet, 3% formaldehyde, 30% ethanol and 0.17% NaCl for 15 min; the wells were then washed exhaustively with water.

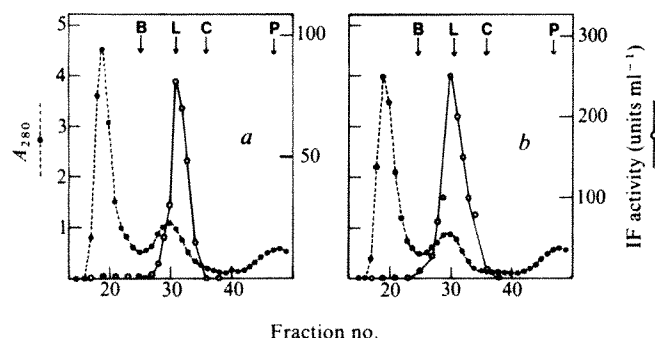


Fig. 2 Chromatography of Le-IF and *E. coli* IF on Sephadex G-100. (a) 0.9 ml S-100 extract of 280-AH3 (500 units, 6 mg protein ml⁻¹) and (b) 0.9 ml of a dilution of human Le-IF (1,000 units of preparation P-IF (ref. 34)) in S-100 extract of SN32 (no IF activity, 6 mg ml⁻¹) were mixed with cytochrome c (0.2 mg) (C), ³²P-phosphate (10⁵ c.p.m.) (P) and ¹²⁵I-labelled β -lactoglobulin (2.5 \times 10⁵ c.p.m.) (L) and chromatographed on a 0.9 \times 49-cm column of Sephadex G-100 in PBS at 4°C. Fractions of 0.7 ml were collected at 2.3 ml h⁻¹. IF activity was measured by the cytopathic effect reduction assay, and radioactivity, A₂₈₀ and A₄₁₀ (cytochrome c) were determined for each fraction. The position of bovine serum albumin (B) was determined in a separate run, relative to C and P.

Table 4 Antibody titres of anti-Le-IF and anti-F-IF measured against different IF preparations

	Le-IF	F-IF	S-100 <i>E. coli</i> extracts	
			SN35	280-AH3
Sheep anti-Le-IF	100,000	3,000	30,000	30,000
Goat anti-F-IF	<10	1,000	<10	<10

About 10 units of each IF preparation were incubated for 1 h at 20°C with different antiserum dilutions and the IF activity was determined by the VSV plaque reduction assay³². The titres are given as the reciprocal of the highest dilution which raises the plaque counts by a factor of 2. The sheep anti-Le-IF was prepared as described elsewhere³³. Human Le-IF was purified to the P-IF stage³⁴.

Human Le-IF is stable at pH 2 (ref. 21). S-100 extracts of 280-AH3 and SN35, as well as 250 units human Le-IF mixed with (inactive) S-100 extract of SN32, were dialysed overnight against 0.1 M NaCl and 50 mM glycine-HCl (pH 2) buffer and then 5 h against phosphate-buffered saline (PBS). A precipitate was removed by centrifugation and the supernatant assayed by both cytopathic effect reduction and plaque reduction. In all cases the initial IF activity was recovered in full.

To compare the MWs of authentic Le-IF and the IF activity in transformed *E. coli* (*E. coli* IF), S-100 extracts were chromatographed on Sephadex G-100 columns. IF activity moved with a K_{av} of 0.46 in the case of 280-AH3 (Fig. 2a), 281-AH6 and SN35 (data not shown), which was slightly slower than authentic Le-IF (mixed with S-100 extract of SN32, Fig. 2b); the difference may, however, not be significant.

We compared the serological properties of authentic IFs and *E. coli* IFs. As shown in Table 4, sheep anti-human Le-IF had a similar titre against Le-IF and *E. coli* IF of SN35 and 280-AH3, and was $\frac{1}{30}$ th as active on fibroblast IF (F-IF); goat anti-human F-IF was active only against F-IF. Thus, *E. coli* IF is immunologically similar to Le-IF, and quite distinct from F-IF.

Both authentic Le-IF and *E. coli* IFs show specificity in regard to the cells on which they will act: they are most active on human cells, less active on monkey and mouse cells and inactive on chick cells (Table 5). It is not clear whether the relatively high activity of *E. coli* IF on monkey cells is significant; further experiments with the purified material are necessary.

As shown by Kerr^{4,5} and others, treatment of cells with IF increases 10- to 15-fold their level of oligoadenylate synthetase, an enzyme that condenses ATP to ppp(A2'p)_n5'A ($n = 1-4$). Cells were treated with various IF preparations and the cell extracts assayed by measuring the ³H radioactivity transferred from ³H-ATP to the dephosphorylation products of pppA2'p5'A and ppp(A2'p)₂5'A, namely (2'-5')ApA and (2'-5')ApApA. As shown in Table 6, (2'-5')ApA radioactivity was six- to ninefold higher in cells treated with Le-IF or *E. coli* IF than in controls treated with an inactive *E. coli* extract, and (2'-5')ApApA radioactivity was more than 14-33 times higher than in controls; there was no significant difference between the activity of Le-IF and S-100 extracts of 280-AH3 and SN35.

Table 5 IF activities measured on different cell types

Cells Expt 1	Le-IF	Interferon activity		
		F-IF	<i>E. coli</i> S-100 extracts	
			280-AH3	SN35
Human U amnion	6,000	2,000	600	600
Monkey Vero	600	600	350	350
Monkey GMK	350	200	350	110
Primary chick embryo fibroblasts	<20	<20	<20	<20
Cells Expt 2	Le-IF	mouse-IF	<i>E. coli</i> S-100 extracts	
			287-AH6	SN35
Human CCL23	1,000	ND	300	1,000
Mouse L929	40	120	40	120

Human Le-IF was preparation P-IF (ref. 34), mouse IF was the NIH standard. U cells were maintained by K.C. All cells were challenged with VSV, except for CCL23 cells, where Mengo virus was used. Experiment 1 was assayed by plaque reduction, experiment 2 by the cytopathic effect reduction assay. ND, Not done.

Thus, by all criteria tested, *E. coli* IF is very similar to authentic Le-IF, although, of course, the molecular structure may well differ in various respects.

Discussion

A strain of *E. coli* containing IF-cDNA was identified by an IF-mRNA hybridisation translation assay in which DNA from successively smaller pools of strains was screened. Because only 4 of 12 groups of 512 clones had originally given a positive response, we were surprised to find 5 IF clones in a selected group of 64. It is probable that, when used on large pools, the assay was at borderline sensitivity and only detected groups and subgroups particularly rich in IF-cDNA clones. The subsequent screening of 5,000 colonies using an IF-cDNA probe revealed 185 positive clones, a frequency of about 1:27. Taking into account the fact that the poly(A) RNA used to generate the clones had been enriched about 10-fold with respect to IF mRNA, the proportion of IF mRNA in poly(A) RNA from induced leukocytes was not less than 1:270.

The identification of the 910-base pair insert in Hif-2h as a cDNA copy of human Le-IF rests on two lines of evidence: (1) its capacity to hybridise selectively to IF mRNA, and (2) its ability to direct the synthesis, in *E. coli*, of a polypeptide with the

Table 6 Levels of oligoadenylate synthetase in human cells treated with Le-IF or *E. coli* IF

Cells treated with:	Cell protein (μg)	³ H-A in oligoadenylate (% of recovered radioactivity)	
		ApA	ApApA
1. S-100 extract of SN35 (200 units IF ml ⁻¹)	7.6	1.4	<0.1
	38	5.2	1.4
2. S-100 extract of 280-AH3 (200 units IF ml ⁻¹)	7.6	1.5	0.1
	38	7.8	3.3
3. S-100 extract of SN32 (no IF)	7.6	<0.1	<0.1
	38	0.9	<0.1
4. Le-IF (P-IF) (200 units IF ml ⁻¹)	7.6	1.3	0.25
	38	8.0	2.1

Confluent CCL23 cell monolayers in 50-mm dishes were treated with a mixture of 1 ml *E. coli* S-100 extract and 4 ml minimal essential medium (MEM)-10% newborn calf serum or a dilution of Le-IF (P-IF) in 5 ml medium. After 20 h the cells were lysed and supernatants prepared as described elsewhere³⁵. Varying amounts of lysate were adsorbed to poly(rI).(rC)-Sephacrose and incubated as described elsewhere³⁵, except that ³H-ATP (specific activity 40 Ci mmol⁻¹) was used instead of ³²P-ATP. After treatment with bacterial alkaline phosphatase, the products were separated by electrophoresis using ApA and ApApA as markers³⁵. The paper was cut into strips and the radioactivity determined by scintillation counting. Most radioactivity was recovered in adenosine. 100% radioactivity was 3-5 × 10⁴ c.p.m.

biological activity of IF. The polypeptide has properties of human Le-IF in that it induces a virus-resistant state in human cells, to a lesser extent in monkey and mouse cells and not in chick cells, and is neutralised by antibody to human Le-IF but not to human F-IF. Moreover, *E. coli* IF stimulates the activity of isoadenylate synthetase in human cells to the same extent as does authentic Le-IF.

The IF-cDNA plasmids were constructed to allow synthesis of an IF molecule fused to part of β -lactamase. It seems likely, however, that the biologically active material is a non-fused polypeptide, because its formation is directed by hybrids derived from each of the three pKT plasmids and is therefore independent of the reading frame resulting from the construction. Moreover, a fused β -lactamase fragment should contribute 180 amino acids when the IF cDNA is inserted in the *Pst*I site of pBR322, but not more than 26 or 29 amino acids when it is linked to pKT280 or pKT287 (K. Talmadge, personal communication); in fact, there is no detectable difference in the size of the biologically active IF polypeptides made by the three strains. At the structural level, *E. coli* IF probably differs from authentic Le-IF by the absence of appropriate glycosylation. Also, it is possible that *E. coli* IF consists of the Le-IF sequence preceded by a signal sequence, as nucleotide sequence analysis of the cloned IF cDNA revealed a region coding for 22 amino

acids which follows the first AUG and precedes the stretch coding for mature IF (M. Schwarzstein, N. Mantei and M.S., unpublished results).

We do not know whether *E. coli* IF has the same specific activity as authentic Le-IF. If this were the case, the amount of active IF produced in transformed *E. coli*, about 20,000 units per l of culture, would correspond to one to two fully active molecules per cell. This would be consistent with the occurrence of rare translational events at the physiological initiation site of the IF sequence, and appropriate modifications of the hybrid plasmid should allow a considerable increase in the yield of active IF. If, however, lack of appropriate glycosylation diminishes the activity of the molecule, we shall have a problem on our hands.

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Note added in proof: Taniguchi and his colleagues have recently identified by nucleotide sequence analysis (T. Taniguchi, personal communication) a fibroblast IF cDNA hybrid prepared from induced fibroblast poly(A) RNA and selected by hybridisation procedures (T. Taniguchi *et al.*, *Proc. Jap. Acad.* **55**, Ser. B, 464–469 (1979)).

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Lethal metabolism of precocene-I to a reactive epoxide by locust corpora allata

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The corpora allata from adult female Locusta migratoria rapidly metabolise precocene-I to a geometric mixture of dihydrodiols, and simultaneously suffer extensive macromolecular labelling. This could explain the selective cell death which precocenes induce, and focuses attention on the high level of epoxidase in these insect organs.

It is not known how the natural product precocene-I (ref. 1), its congener precocene-II (ref. 1), or related analogues², cause selective necrosis^{3,4} of cells in the corpora allata (CA) of sensitive insects. Therefore, it has not been possible to rationalise the activity or inactivity of precocene analogues², or to explain the marked species specificity of these compounds. An early suggestion⁵ that precocenes might act on the brain, which normally controls the CA, has received no further experimental support. Initial experiments on the isolated CA of a relatively insensitive species were inconclusive⁶. More positive evidence for a direct action of precocenes on the CA comes from studies on CA of adult female *Oncopeltus*⁷, in which 87% of CA failed to elicit a positive response in a transplantation bioassay after culture for 5 days in a medium containing 4.5×10^{-5} M precocene-II. The likelihood of an oxidative biotransformation being crucial to the action of precocene was raised by our recent finding⁸, that its action on both *Oncopeltus* and *Locusta* is suppressed by co-treatment with certain benzodioxole mixed function oxidase inhibitors, although these seem to protect precocene against metabolic inactivation. We speculated⁸ that a crucial activating metabolism of precocenes may occur within the CA, which might explain their tissue specificity. Accordingly, we have synthesised radiolabelled precocene-I, and

studied its metabolism by CA and non-target tissues of adult female *Locusta migratoria*, *in vitro*. Our results strongly indicate that the CA rapidly oxidise precocene-I at the 3,4 double bond to a highly reactive⁹ epoxide, much of which forms covalent attachment to cellular macromolecules. We suggest that this may be the basis of the cytotoxic action of precocenes. By contrast, non-target tissues are not subjected to intense labelling of macromolecules, which may be important in determining the selective action of precocenes. Our hypothesis focuses attention on the enzymic competence of the CA to oxidise precocene-like molecules to highly reactive epoxides.

Precocene metabolism and covalent attachment to macromolecules

We synthesised [4-³H]precocene-I (2,2-dimethyl-7-methoxychromene) at 34 mCi mmol¹⁰. Authentic reference compounds I–IV (Fig. 1) were prepared for identification of possible metabolites by published methods^{1,9,11}, all being known compounds. 2,2-Dimethyl-7-hydroxy-chromene¹² was prepared by the method of Bowers *et al.*¹ from the corresponding chromanone¹¹. The dihydrodiols (IV)¹³ were prepared from the *m*-chloroperoxybenzoic acid adducts to precocene-I (ref. 9) after separation of *cis* and *trans* forms (Waters Prep system 500,

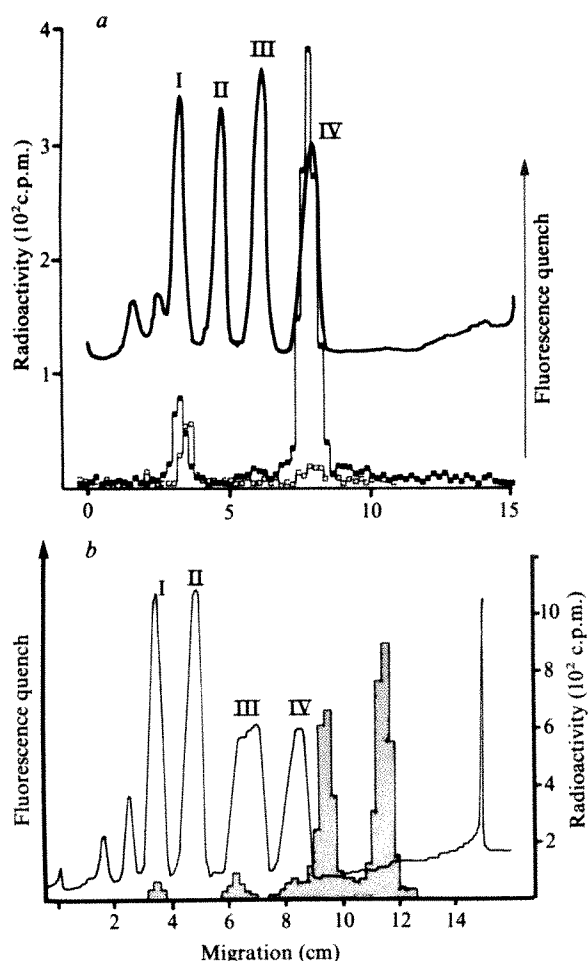


Fig. 1 Radio-TLC analysis of precocene-I metabolites produced by several locust tissues *in vitro*. *a*, Five lots of two pairs of CA from 14-day-old adult female *Locusta migratoria*, reared as described elsewhere⁸, were incubated by shaking in the dark at 30 °C in 0.1 ml each of TC 199 medium (2 mg ml⁻¹ FicollTM, 20 mM HEPES buffer, pH 7.2 at 30 °C) with [4-³H]precocene-I (34 mCi mmol⁻¹; 99.3% radiochemical purity by quantitative RP-TLC) at an initial concentration of 0.101 mM, for 3 h. Incubation tubes and glass transfer pipettes were coated with 5% aqueous Carbowax 20 M. The incubation was stopped by exhaustive extraction on the centrifuge with ethyl acetate (containing 20–50 µg nonradioactive reference compounds) the extract dried with anhydrous sodium sulphate, and the volume reduced under nitrogen flow before TLC application. RP-TLC was carried out on Merck 0.25 mm KC-18 indicator plates, pre-washed in alumina purified ethyl acetate then AR methanol, after application to 1.5 × 1.5-cm area. The samples were prefocused twice with redistilled methanol then developed, in a darkened sandwich chamber for 15 cm in methanol:water (7:3). Authentic internal reference compounds were localised by fluorescence quenching densitometry (continuous line) (Vitatron TLD), and radioactivity localised by windowless gas flow counting (Berthold scanner, 1-mm window, scaler mode, background 3 c.p.m.). Reference compounds: I, 2,2-dimethyl-7-methoxy-chromene (precocene-I); II, 2,2-dimethyl-7-methoxy-chroman-4-one; III, 2,2-dimethyl-7-hydroxy-chromene and 2,2-dimethyl-7-methoxy-chroman-4-ol (poorly resolved); IV, *cis, trans* 3,4-dihydroxy-2,2-dimethyl-7-methoxy-chroman (precocene-I dihydrodiols). ■, CA incubation; □, control incubation without tissue. *b*, Similar experiment to *a*, using Malpighian tubules, except that the initial precocene concentration was 0.14 mM, and the combined incubations were blended in 5 ml of redistilled ethyl acetate before repeated extraction. Qualitatively similar results were obtained with ligatured mid-guts and abdominal fat body sheet.

two series mounted silica PrepPAKs 5.7 × 30 cm, 30% diethyl ether in pentane) by base hydrolysis (1M NaOH, 50% aqueous ethanol) 30 min at room temperature). All compounds were characterised by appropriate physical methods. When CA from reproductively mature female *Locusta* were incubated in tissue culture medium containing labelled precocene-I for 1–5 h, and the incubations partitioned between either salt solution/chloroform + methanol, or /ethyl acetate, negligible radioactivity (<1.5%) was found in the aqueous phase. Analysis of the chloroform layer by reversed-phase thin layer chromatography (RP-TLC) suggested that there was only one important metabolite (96% of products) in the CA, which co-chromato-

graphed with a mixture of *cis/trans* 3,4 dihydrodiols, whereas the selected non-target tissues (abdominal fat body, ligatured mid-gut and Malpighian tubules) produced varying quantities of material of intermediate polarity (Fig. 1). In two experiments, half the chloroform layer was also analysed by normal phase TLC (silica gel; ethyl acetate: xylene, 25:75) with mutually consistent results (data not shown). When the CA were separated from the incubation medium at the end of the experiment by gland transfer¹⁴, and extracted separately, more than 99% of the dihydrodiol present was found to be in the surrounding medium. Analysis of the extracted CA revealed up to 400 pmol precocene equivalents per gland pair bound to non-extractable residues, whereas the radioactivity associated with non-extractable residues from the non-target tissues was much less (~4–15 pmol per animal equivalent) after 3 h incubation. In three experiments, duplicate CA samples were subjected to further extraction with 10% TCA before solubilisation and radioactivity determination, with only slight (1–8%) loss of radioactivity. We take this to be evidence of covalent attachment, but have yet to demonstrate either its nature or cellular location. Depending on the initial concentration of precocene in these experiments (range 1.7×10^{-5} – 4.4×10^{-4} M) CA macromolecules were labelled to the extent of 7.8–80 nmol per mg tissue protein. These are very high values. For example, the lowest value is 80 times the degree of labelling of rat liver protein *in vivo* following administration of an hepatotoxic dose of bromobenzene, even when protection by endogenous glutathione is at its minimum¹⁵.

We studied the time course of precocene–dihydrodiol formation and macromolecule labelling in the CA by replacing the substrate medium every 30 min (Fig. 2a), thereby maintaining the precocene concentration within a defined range. Figure 2a shows a trend towards progressively increased rate of dihydrodiol release over a period of 2.5 h. Macromolecules are labelled at a constant rate in these conditions, after an extrapolated lag period of 9 min. We also examined the time course of metabolism during incubations in which an initially high precocene concentration was allowed to decline exponentially for 3 h. Figure 2b shows one such experiment, resulting in a final concentration of 7×10^{-5} M, and it is clear that dihydrodiol formation and macromolecule labelling proceed unabated over this period. Other experiments showed that the metabolic rate varies with, but is not proportional to, the precocene concentration over the range studied.

Stereochemistry of precocene dihydrodiols

To identify the dihydrodiols further, we isolated 2.9×10^5 d.p.m. by RP-TLC, and analysed aliquots by high resolution silica HPLC (Fig. 3). Of the radioactivity recovered (94% of that applied), 96% co-chromatographed exactly with authentic internal markers of *cis* and *trans* dihydrodiols, with radioactivity in the ratio *trans*:*cis*, 62:38. We also examined the very much smaller quantities of dihydrodiol produced by the three other tissues studied, where it does not accumulate progressively, and obtained *trans*:*cis* ratios of 63:37 to 80:20.

We believe that the high *cis* content of the dihydrodiol produced by the CA is important evidence of trigonal hybridisation in a reaction intermediate, namely the 3-OH, 4-carbocation which would result from proton catalysed opening of the 3,4 epoxide ring; it is known that chemical hydration of precocene-I 3,4-epoxide yields significant quantities (21–36%) of *cis* dihydrodiol in a wide variety of conditions (ref. 9 and A. F. H., A. P. Ottridge, G. E. P., R. C. J. and K. M. Stott, unpublished results). This proposed hydration mechanism, and the high reactivity of precocene-I epoxide⁹, compares readily with that deduced for the reactive epoxides of benzo(α)pyrene 7,8-dihydrodiol¹⁶ and many other mammalian carcinogens¹⁷. This contrasts markedly with the relatively stable epoxide group in the natural product (juvenile hormone) of the CA, whose acid-catalysed hydration is predominantly S_N2 in character and leads to 97% *trans* dihydrodiol¹⁸. Enzymic hydrolysis of epoxides typically yields *trans* dihydrodiols¹⁹. Apparently, dihydrodiols

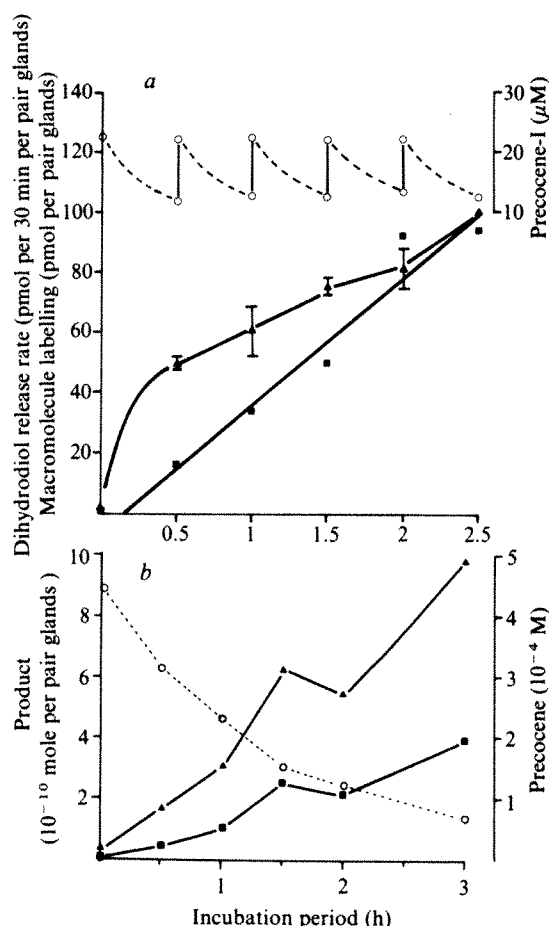


Fig. 2 Progressive formation of dihydrodiols and labelled macromolecules from [4-³H]precocene-I by *Locusta* CA, *in vitro*. Glands were incubated as in Fig. 1, but at different precocene-I concentrations. Δ , Incubations were extracted with 25 volumes of CHCl₃:methanol (10:1) and the extracts analysed by RP-TLC for precocene-I dihydrodiol content. \blacksquare , Labelled macromolecules were estimated after removal of the CA from the aqueous phase on stainless steel loops and extraction twice in 0.5 ml methanol (6 h), before solubilisation (0.2 ml Lumasolve, LKB, 2 h, 45 °C with shaking) and liquid scintillation counting (4.7 ml Lumagel, LKB, neutralised with glacial acetic acid, shaken for 1 h) in a Wallac 81000 calibrated on AES ratio with standard tritiated water (Amersham), in minivials. Radioactivity on RP-TLC plates was qualitatively localised by gas-flow β -imaging (Panax Betagraph) and correlated with the fluorescence quenching profile of authentic *cis/trans* precocene-I dihydrodiols, which were recovered by scraping into micro-filter tubes under suction, and quantitatively eluted with 8 \times 0.2 ml methanol into 9.5 ml of toluene-based scintillator²⁵ for scintillation counting. *a*, CA were bilaterally randomised between five incubation tubes (four glands per 0.1 ml culture medium) and incubated for different total periods, in duplicate, in 2.3×10^{-5} M [4-³H]precocene-I. The medium was recovered by aspiration every 30 min, for analysis by RP-TLC, and replaced with fresh medium, until the end of the experiment. The degree of cumulative labelling of cellular macromolecules was determined at the end of each incubation; each point represents the mean of two separate observations, and the straight line is a fitted linear regression ($r = 0.96$). The stock solution of substrate in TC 199 was stable at 0 °C. \circ , The concentration of precocene-I at the end of each 30-min interval was determined by subtracting the radioactivity found in the dihydrodiol zone after RP-TLC, from the total radioactivity in the medium (determined by scintillation counting of a 5- μ l aliquot of a 1:1 mixture and ice-cold methanol prepared immediately after aspiration of the medium from the CA). The points are variously the means of 2–10 estimations, and standard errors are indicated where appropriate. The saw-tooth line joining the concentration points is purely illustrative (compare with *b*). The final balance of radioactivity was completed by elution of the plastic incubation caps in toluene (10% ethanol) scintillant. *b*, Similar to *a*, except that the incubations were initiated at 4.45×10^{-4} M [4-³H]precocene-I, and the media were not replaced during the course of the incubations. Each point is the mean of two determinations.

are important metabolites of precocene-II in whole insects of several species²⁰, and in an isolated non-target tissue (fat body) from two insensitive species²¹, but their stereochemistry has not been reported. We have used standard methods²² to investigate epoxide hydrolase activity in isolated *Locusta* CA with [12-³H]C₁₆ juvenile hormone (340 mCi mmol⁻¹) as substrate, and

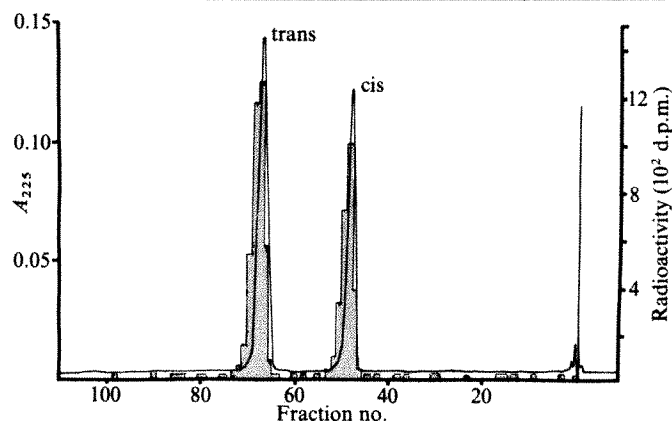


Fig. 3 High resolution liquid chromatograph of precocene-I dihydrodiols produced by *Locusta* CA *in vitro*, and isolated by RP-TLC. Radioactivity in the dihydrodiol zone after RP-TLC (Fig. 1a) was eluted with a total of 1.5 ml redistilled dichloromethane, filtered and evaporated. The sample was dissolved in HPLC solvent, and 4.8 μ l (6,914 d.p.m.) analysed on 5 μ m Zorbax-sil (250 \times 4.6 mm; diethyl ether:pentane:2-propanol (49.9:49.9:0.2), 1.5 ml min⁻¹). Fractions of effluent were collected every 12 s for determination of radioactivity.

failed to find any (detection limit ~ 1 pmol h⁻¹ per gland pair), which is in accord with the physiological function of these glands. Thus, if dihydrodiol formation in the CA is a consequence of epoxidation, the final hydration step is almost certainly a spontaneous chemical event, proceeding via a reactive carbocation which would be expected to alkylate cellular nucleophiles^{9,15} in competition with its reaction with water. Apparently, our proposed pathway may account for both the stereochemistry of the dihydrodiols and the labelling of cellular macromolecules.

Sensitivity to a mixed function oxidase inhibitor

We sought evidence for an initial lethal synthesis involving epoxidase action on precocene-I by studying the effect of a mixed function oxidase (MFO) inhibitor on precocene metabolism in the CA. Many MFO inhibitors are known to inhibit juvenile hormone (JH) biosynthesis in both whole cockroach CA²³ and homogenates thereof²⁴. As part of a larger study

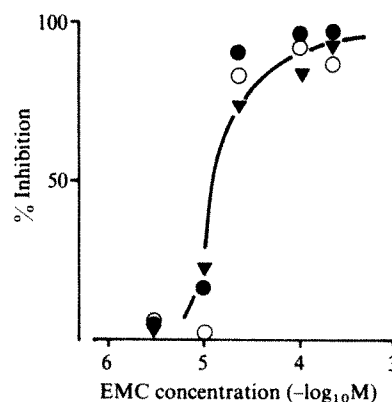


Fig. 4 Inhibition of precocene-I and farnesenic acid metabolism in *Locusta* CA by EMPB, *in vitro*. CA were bilaterally randomised between control and experimental tubes, and pre-incubated with the appropriate concentration of EMPB for 30 min before addition of radiolabelled substrates²³. When precocene-I was the substrate it was present at an initial concentration of 0.12 mM, and radiolabelled incubation was for 2 h. Precocene-I (\bullet) dihydrodiols and labelled macromolecules (\circ) were determined as described in Fig. 2 legend. When farnesenic acid was the substrate the media contained [12-³H]2E,6E-farnesenic acid (309 mCi mmol⁻¹; 2.6×10^{-5} M), and [Me-¹⁴C]methionine (Amersham; final activity 35.4 mCi mmol⁻¹; 1.6×10^{-4} M). Methyl farnesate and juvenile hormone (∇) were quantified by extraction, silica gel TLC and liquid scintillation counting²⁵. Inhibition values were the same whether calculated on ³H or ¹⁴C radioactivity. The data are composed from two separate experiments, each point representing a single observation except those at 10⁻⁴ M EMPB, which are duplicates.

(G.T.B., G.E.P. & J. A. Cocks, unpublished) we synthesised ethyl (2*E*)-4-(3,4-methylenedioxyphenoxy) butanoate (EMPB) by transesterification (BF₃/ethanol) of the corresponding methyl ester derived from the esterification of sesamol with methyl 4-bromo-2*E*-butanoate (K₂CO₃/dimethoxyethane, 60 °C). The compound was 98% pure by HPLC (5 µm Zorbax-sil, *N*_{eff} = 9,300, pentane: diethyl ether: s-propanol 9.9: 89.9: 0.2). We tested this compound in a standard assay for inhibition of spontaneous C₁₆H biosynthesis in cockroach CA²³, and found an IC₅₀ of 8 × 10⁻⁶ M coupled with a large accumulation of methyl farnesoate, unlike some other MFO inhibitors which seemed to inhibit both epoxidation and esterification reactions in the biosynthetic pathway²³. EMPB also inhibits C₁₆H biosynthesis from exogenous farnesenic acid²⁵ in *Locusta* CA (Fig. 4), although it seems to be slightly less active in this system. More importantly, Fig. 4 shows that adult female *Locusta* CA are almost equally sensitive to EMPB in respect of: (1) epoxidation of internally generated methyl farnesoate; (2) precocene-I dihydrodiol formation; and (3) precocene labelling of macromolecules. We observed no significant diversion of precocene-I metabolism towards other products in the presence of EMPB. These results demonstrate that an oxidative enzyme is crucial to both ultimate pathways of precocene metabolism in the CA. Kinetic studies on a partially purified methyl farnesoate epoxidase from adult female *Locusta* CA have revealed high

levels of activity, with typical *v*_{max} values of 1–2 nmol min⁻¹ per mg total tissue protein (R. Feyereisen & G. E. P., unpublished results). We have yet to demonstrate that precocene-I is a lethal alternative substrate for this enzyme, but suspect this to be the case, because the specialised endocrine function of the glands makes it unlikely that they will be rich in other oxidases involved in general detoxifying metabolism.

Conclusions

The oxidative activation of precocene-I in *Locusta* CA may be compared with the various types of oxidation which are believed to be crucial to the action of aromatic cytotoxins¹⁵, neurogenic neurotransmitter analogues²⁶ and carcinogenic polycyclic aromatic hydrocarbons^{16,17} in vertebrate tissues. In these cases, the mechanisms of possible importance include the generation of epoxides^{15–17}, quinonoids^{26,27} and reactive forms of oxygen^{26,28}. Causative interpretation of these findings has been particularly difficult in the case of polycyclic aromatic hydrocarbons because of the multiplicity of metabolic pathways²⁹. In contrast, the present studies suggest that precocenes are metabolised by a single enzymic pathway in the CA; which should facilitate investigation of their selective cytotoxic action.

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LETTERS

Emission at 3.3 µm and evidence for dust in 3C273

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The presence of dust grains in a variety of astronomical environments has been established from many IR observations¹. Of particular interest is whether dust exists in the highly energetic quasars, and in the less energetic (but apparently similar) Seyfert 1 galaxies. Whilst both quasars and Seyfert 1 galaxies exhibit enhanced radiation over just the wavebands expected of thermal dust emission, the interpretation of this enhancement is a matter of controversy². The origin of the non-thermal radiation that quasars emit at other wavebands is not understood; hence a non-thermal origin of their enhanced IR continua cannot be excluded. The existence of dust also has relevance to current discussions on the hydrogen emission-line ratios, for the weakness of the broad Lyα emission can possibly be explained by absorption on dust grains^{3,4}. I present here circumstantial evidence that dust exists in the quasar 3C273, by demonstrating the existence of the 3.3 µm line in emission.

Emission at 3.3 µm has been found in many galactic and extragalactic objects. In the most complete reviews of this feature^{5,6}, the wavelength was given as 3.28 µm, and a qualitative correlation with the presence of dust grains was noted. A gas-phase origin of the emission was shown to be unlikely because of the intrinsic width of the feature⁵. Recently an identification with C–H bonds (for example, methane) on the surface of dust grains has been proposed⁷.

Spectroscopic observations were made of the quasar 3C273 using the IR photometer-spectrometer on the 3.9 m Anglo-Australian Telescope. In its spectroscopic mode this instrument makes use of a circular variable filter and is a single-channel fast-scanning spectrometer. A total of 1,800 scans through the spectrum were made in a period of 2.5 hours; the spectral region covered was 3.68–3.96 µm. The spectral resolution was 0.08 µm, and samples were taken every 0.02 µm. For the redshift of 3C273 the expected wavelength of the 3.28 µm feature is 3.80 µm.

The data are presented in Fig. 1. An unresolved emission feature is clearly seen rising about 40% above the continuum and having an equivalent width of 170 ± 40 Å. The rest wavelength of this feature is 3.26 µm. This differs from the observed wavelength of the feature in other objects by 0.3 of the spectral resolution, an amount roughly equal to the uncertainty of the wavelength calibration in this spectral region.

The presence of the 3.28-µm feature is strong circumstantial evidence for the existence of dust somewhere in the immediate

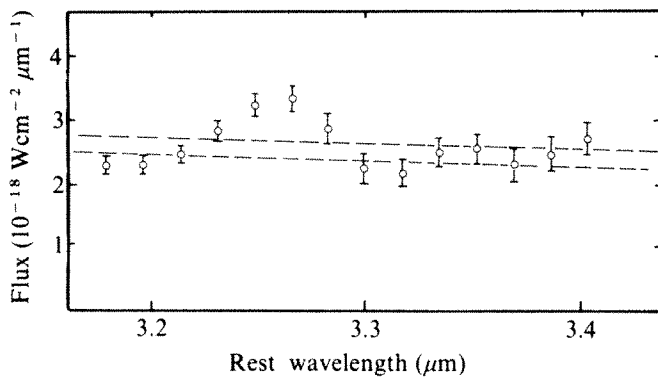


Fig. 1 Spectrum of 3C273 in the rest wavelength range 3.2–3.4 μm . The two broken lines indicate the 1- σ bounds to the estimated continuum based on independent broad-band IR measurements made on the same night (9 April 1979).

vicinity of 3C273. This in turn makes more credible the association of the IR enhancement with thermal radiation from dust. Whether that dust lies within the emitting gas, and therefore can absorb a significant fraction of the Ly α emission, cannot be determined from present data. If, however, the 1–10 μm continuum⁸ in 3C273 is interpreted as thermal radiation from

that dust, the black-body colour temperatures implied range from a few hundred to about 1,000 K. Such temperatures would be found at distances of the order of 10 to 100 pc from the central energy source for a luminosity of about $10^{13} L_{\odot}$ (ref. 8). The dust therefore lies outside at least part of the nuclear region wherein the broad emission lines probably originate. In a study of the effects of dust on the emission-line spectra of quasars, Ferland and Netzer⁹ concluded that the weakness of Ly α could possibly be explained better by dust outside the emission region than within it. However, such statements are inevitably provisional as there is no guarantee that the dust is distributed with spherical symmetry about a quasar's nucleus.

It would be of interest, although difficult, to search for the emission features at 7.8 and 8.7 μm , which are present in most galactic sources having 3.3- μm emission⁶, and which are redshifted to 9.0 and 10.1 μm , both convenient parts of the atmospheric window.

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A probable 1970 hard X-ray outburst by 4U0041+32

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During a balloon test flight on 4 February 1970, an engineering model of the UCSD Cosmic X-ray Experiment, later flown on the OSO 7 satellite, observed what was apparently an unknown high-latitude X-ray source at $\alpha = 0^{\text{h}} 45^{\text{m}} \pm 5^{\text{m}}$, $\delta = 33^{\circ} \pm 3^{\circ}$. The source had a 25–300 keV flux of about 6×10^{-2} photons $\text{cm}^{-2} \text{s}^{-1}$, and a very hard spectrum over that energy range. By the time of a subsequent balloon flight on 25 September 1970, the intensity had faded to below 10^{-2} photons $\text{cm}^{-2} \text{s}^{-1}$ (99% confidence). Recent observations by several experimenters have led us to associate the balloon observation with the highly variable source 4U0041+32. Certain characteristics of this source, such as its high galactic latitude and hard spectrum extending to ~ 300 keV, set it apart from other known transients.

At the time of the 1970 balloon flights there was no known source in the region. The 2U Catalogue¹ of 1972 contained an entry, 2U0043+32, compatible with the balloon position, but the source was weak (7.8 ± 0.6 Uhuru counts) and not described as variable. Variability was first suggested by MIT OSO 7 data², and confirmed by Murray and Ulmer³ in 1976, based on further analyses of Uhuru data. In 1977 February an X-ray outburst⁴ occurred, during which the source reached an intensity of ~ 50

Uhuru counts. SAS 3 observations⁵ over the following several months improved the source position to a 1 arc min radius error circle, allowing a tentative optical identification by Charles *et al.*⁶. The identification was based on weak He II $\lambda 4686$ emission seen on one of several nights' spectrophotometric observations of the $V \sim 19.2$ mag G-type candidate star. The faintness of the candidate (and the absence of bright stars nearby) seems to require a distance of at least 5 kpc, placing the high latitude object within the galactic halo. Evidence for a possible 11.6-day periodicity has been reported by Watson and Ricketts⁷ from Ariel 5 data, while the SAS 3 observations⁵ limit short-period pulsations to $< 7\%$ of the total flux. The aforementioned discoveries, together with others establishing the hard X-ray transients as fairly common phenomena^{8–12}, have now made it clear

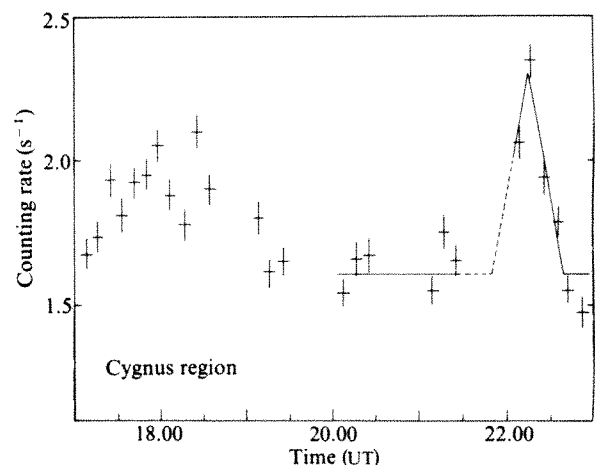


Fig. 1 20–50 keV counting rate versus time during the February 1970 balloon flight. The solid line shows the expected collimator response to a point X-ray source at $\alpha = 0^{\text{h}} 45^{\text{m}}$, $\delta = +33^{\circ}$. The high rates early in the flight are due primarily to Cygnus X–1. Gaps in the data indicate times when the instrument was being calibrated or when the blocking crystal was in the detector aperture.

that the 1970 balloon observation was probably of a prediscovery outburst of 4U0041+32.

The observation was made by a scintillation telescope¹³ consisting of a 64 cm² area, 1-cm thick NaI(Tl) central detector collimated to a $\sim 7^\circ$ FWHM field-of-view by a surrounding CsI(Na) shield in active anticoincidence. As the flight was primarily for test purposes, the instrument was mounted vertically beneath a rotating table that contained an opening, a calibration source, and a NaI blocking crystal. To obtain the maximum amount of diagnostic information, individual central detector events in the 15–550 keV range were pulse-height analysed in a 256-channel linear PHA and telemetered to the ground along with associated shield coincidence tags. The actual anticoincidence vetoing was carried out during subsequent computer analysis. In addition, nearly all central detector and shield discriminator rates and many other housekeeping functions were continuously monitored. Background spectra and calibrations were taken hourly utilising the rotating table, except during the early part of the flight when the Cygnus region crossed the detector aperture.

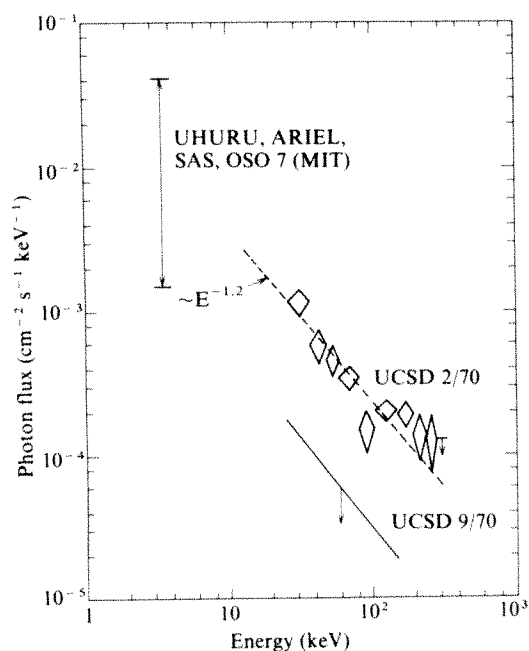


Fig. 2 Spectral Information on 4U0041+32. The range of intensities seen in the ~ 2 –10 keV range is shown together with the two 1970 balloon observations.

Figure 1 shows 20–50 keV counting rates observed during the flight. The low rates reflect post-flight processing to include the effects of the anticoincidence shielding. A source transit apparently occurred shortly after 22.00 UT. Although the data were never published (due, among other reasons, to the second flight's failure to confirm the source), the apparent transit has all the characteristics expected for a real cosmic X-ray source. First, the hard spectrum of the excess counts (Fig. 2) rules out several alternative possibilities, such as an increase in detector (PMT) noise, an X-ray aurora, or most precipitating particle events. Second, there was no significant increase or decrease in any of the instrument discriminator counting rates during the transit. Possibilities such as a change in anticoincidence efficiency due to degraded shield performance, or any increase in the overall radiation environment, are, therefore, largely excluded. Furthermore, the radiation causing the excess counting rate must have formed a well-collimated beam, because the shield rates, which are sensitive to diffuse or isotropic radiation but relatively insensitive to unidirectional radiation beams, were also constant within the statistical uncertainties. As it is highly

implausible that charged particles could form such a beam and maintain it stably over the ~ 30 min duration and ~ 50 km spatial extent (the balloon drift rate was ~ 100 km h⁻¹) of the observation, the radiation beam must have been a beam of photons—an X-ray source. Finally, the time history of the excess counting rate (Fig. 1) agrees well with that expected from the collimator response to a point source at $\alpha = 0^{\text{h}} 45^{\text{m}} \pm 5^{\text{m}}$, $\delta = 33^\circ \pm 3^\circ$. If we use the scatter in the eight background data points between 20.00–21.30 UT and 22.30–23.00 UT to determine the errors (thereby obtaining errors about 30% larger than those expected from Poisson statistics), the 20–50 keV source transit shown in Fig. 1 has a significance of $\sim 10\sigma$.

Given the moderate sensitivity of our balloon experiment, it seems unlikely that we have obtained the only observation of a high latitude ($b \sim -30^\circ$) variable or transient X-ray source located within 3° of the known variable 4U0041+32. Unfortunately, our incomplete knowledge of the occurrence of this type of object, does not allow us to place a reliable quantitative value on the probability of such a coincidence. Note also that the weak (3 Uhuru counts s⁻¹) source 4U0041+36 could conceivably have been responsible for the 1970 outburst. Nonetheless, as it seems most likely that the balloon source and 4U0041+32 are the same, we shall base the following discussion on that assumption.

The spectrum that we obtained for 4U0041+32 is shown in Fig. 2, along with the 1970 September upper limit and with data from the 1–10 keV observations. While simple power law extrapolations of the high energy data seem generally consistent with those taken at lower energy, the absence of simultaneous measurements over the entire 1–300 keV range prevents us from determining whether spectral changes accompany the intensity variations. In any case, the fact that the spectrum extends to 300 keV, apparently without a break, sets 4U0041+32 apart both from the other hard transients and also from the hard compact binary X-ray sources, which generally have a spectral cut-off below a few tens of keV. The spectrum is similar to, but harder than, spectra of some compact extragalactic sources (for example, Cen A, NGC4151, and 3C273; see refs 14–16) and most resembles the spectra of certain cosmic γ -ray bursts (for example, 14 May 1972; see ref. 17). The source is distinguished from the other hard transients not only by its lack of a high energy spectral cut-off, but also by its position (>2.5 kpc below the galactic plane), by its apparent lack of pulsation⁵, and by not being associated with an early type giant or supergiant companion. Assuming a distance of 5 kpc, the peak X-ray luminosity of $\sim 3 \times 10^{37}$ erg s⁻¹ is in the range observed for other hard transients, and for the hard compact binary X-ray sources.

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Can pregalactic black holes produce the hard X-ray background?

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The possibility that pregalactic black hole accretion could have heated the Universe sufficiently to produce the hard X-ray background through bremsstrahlung emission is considered here. This would only be possible if the holes were implausibly large. However, the radiation produced directly from the accretion could explain both the density and spectrum of the X-ray background providing that the holes had a density of the order of 10^{-6} times the critical density and a mass of the order of $10^9 M_\odot$. We suggest that these holes could be the precursors of the holes which may presently be powering Seyfert galaxies or quasars.

It has been claimed¹ that the spectrum of the extragalactic diffuse X-ray background from 3 to 50 keV is well described as optically thin bremsstrahlung radiation with a temperature of ~ 45 keV. The energy density of this background, $\Omega_x \sim 10^{-8}$ in units of the critical density, could conceivably be generated by known discrete sources² (such as quasars). However, it would be surprising if the superposition of many discrete sources, each presumably having a different spectrum, could produce the very specific background spectrum required, and this suggests³ that the X-ray background may be produced by a uniform intergalactic medium with density $\Omega_g \sim 0.5$ and temperature 5×10^8 K. This would naturally explain why the background should have an optically thin bremsstrahlung spectrum. If such a medium does exist, however, it is hard to explain how it became so hot. Models which invoke quasar heating face severe energetic problems and, although these problems are alleviated if one assumes that the medium is clumped into large clouds, one would then expect anisotropies in the X-ray background larger than is observed⁴.

We first consider whether the intergalactic medium could have been heated to 5×10^8 K as a result of pregalactic black hole accretion. This suggestion is prompted by the realisation that black holes would have been accreting very rapidly at pregalactic epochs and that, if they produced radiation with an appreciable efficiency, they would have inevitably boosted the matter temperature well above the usual Friedmann value, generally causing reionisation. There are many contexts in which a population of black holes could have formed before galaxy formation. They could have been produced even before decoupling if the early Universe had large density fluctuations⁵ or a soft equation of state⁶ or it was highly anisotropic⁷ or cold⁸ or tepid⁹. More conventionally, one might expect holes to form after decoupling when the Jeans mass drops to $10^6 M_\odot$ and regions that size should bind very easily; such regions might either collapse directly or fragment into massive (10^2 – $10^4 M_\odot$) stars which could themselves collapse after their brief nuclear-burning phase of 10^6 yr. Within all these contexts one would expect the holes to have a mass above $10 M_\odot$ and their density Ω_B could, in principle, be close to 1. In this case, they would be good candidates for providing the 'missing' mass in clusters and galactic haloes¹⁰. If their density was very small, they might be identified with the black holes which are suspected of residing in quasars and active galactic nuclei¹¹.

The effect of pregalactic black-hole accretion on the thermal history of the Universe has been studied in detail elsewhere¹².

Here we outline the argument for determining the maximum temperature attained. We assume that the holes are subsonic at pregalactic epochs and that they accrete at the Bondi¹³ rate $\dot{M} \sim 10^{11} M^2 n T_4^{-3/2} \text{ g s}^{-1}$, where M is in solar masses and n and T_4 are the background particle density in cm^{-3} and temperature in units of 10^4 K. (For simplicity we assume a critical background density; in this case $n \sim 10^{-5} z^3$ for $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$.) If the accretion generates radiation with an efficiency ϵ , then holes larger than $10^3 \epsilon^{-1} M_\odot$ will be accreting at the Eddington limit for some period after decoupling and during this 'Eddington phase' we assume such holes radiate at the Eddington limit $L_{\text{ED}} \sim 10^{38} \text{ Merg s}^{-1}$.

We need to know how much of the radiation goes into heating the Universe and how much goes into the background. Photons emitted at a redshift z will go into Compton heating providing their energy exceeds about $10 z^{-3/2} \text{ MeV}$. Thus if most of the radiation is at an energy $E_{\text{max}} = 10 \eta \text{ keV}$, nearly all of it will go into Compton heating at redshifts exceeding $z_* \sim 10^2 \eta^{-2/3}$; the fraction doing so thereafter is just $f_c \sim (z/z_*)^{3/2}$. Photons with energy $\leq z^{1/2} \text{ keV}$ will go into photoionisation heating. If most of the emergent radiation is at an energy higher than this, the fraction which goes into photoionisation heating will be small; for a flat spectrum this fraction is $f_p \sim \eta^{-1} (z/10^3)^{1/2}$.

For even very small values of the black hole density Ω_B one can show that the photoionisation heating will cause the matter temperature to rise at some time after decoupling and eventually reach a value of $\sim 10^4$ K. At this point the ionisation will increase to a value close to 1 due to collisions. Because of the inverse Compton cooling of the 3 K background,

$$\Lambda_r \equiv -\left(\frac{dT}{dt}\right)_r \approx \frac{\sigma_T \rho_r T}{m_e c} \approx 10^{-19} T z^4 \text{ s}^{-1} \quad (1)$$

(where ρ_r is the 3 K radiation density, σ_T is the Thomson cross-section and m_e is the electron mass), T cannot initially rise above 10^4 K. However, it will do so when the Compton heating of the holes

$$\Lambda_c \equiv \left(\frac{dT}{dt}\right)_c \approx \frac{n_B L_{\text{ED}} f_c}{nk} \approx 10^{-3} \Omega_B \text{ K s}^{-1} \quad \text{for } z > z_* \quad (2)$$

(where n_B is the black hole number density) becomes comparable to Λ_r . This occurs at a redshift $z_c \sim 10^3 \Omega_B^{1/4}$. Thereafter T , determined by the balance of Λ_c and Λ_r , rises as z^4 until the accretion rate falls below the Eddington limit at a redshift which can be shown to be

$$z_{\text{ED}} \approx 10^{3.3} \Omega_B^{1/6} (M\epsilon)^{-1/9} \quad (3)$$

(This exceeds z_* for the values of Ω_B and M which are of interest.) After the Eddington phase, Λ_c is reduced and T rises as $z^{-2/5}$ for $z > z_*$ and falls as $z^{1/5}$ for $z < z_*$. At a redshift of the order of 10 the Compton heating time scale falls below the expansion time scale, so T begins to fall faster. However, at about this time the thermal history may be affected by various postgalactic processes (such as quasar heating³), so that our analysis breaks down.

These considerations imply that the maximum temperature is attained at the redshift z_* when f_c falls below 1 and it is

$$T_{\text{max}} \approx 10^{3.2} (\Omega_B M \epsilon)^{2/5} \eta^{4/15} \text{ K} \quad (4)$$

providing this does not exceed the temperature of the hottest accretion-generated photons $\sim 10^8 \eta \text{ K}$. Our estimate of T_{max} is insensitive to the spectrum of the emitted radiation so long as most of the energy is at the upper end (that is, as long as the spectrum is shallower than E^{-1}). Clearly, T_{max} can only be as high as 10^8 K , say, if the holes are very large indeed. Even with the optimal assumptions ($\Omega_B \sim 1$, $\epsilon \sim 0.1$, $\eta \sim 10^3$) one requires M to exceed $10^{12} M_\odot$ and there are independent limits which preclude holes of this size from being very numerous¹⁰. It is,

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therefore, implausible that pregalactic black hole accretion could heat the Universe to the temperature required to explain the hard X-ray background, even though one would expect it to reionise the Universe ($T_{\max} > 10^4$ K).

We now consider whether the fraction of generated radiation which does not go into heating the Universe could explain the 45-keV background. After the redshift z_* , this fraction is essentially unity, whereas before z_* it is only $(z_*/z)^{3/2}$. It is easy to deduce¹² that the largest contribution to the background density derives from the epoch z_* and that the redshifted energy generated by each hole is

$$E(M) \approx (\epsilon \dot{M} c^2 z^{-1} t)_* \approx 10^{46} \Omega_B^{-3/5} M^{7/5} \epsilon^{2/5} \eta^{-11/15} \text{ erg} \quad (7)$$

This corresponds to a present background density

$$\Omega_x \approx n_B E(M) / \rho_{\text{crit}} \approx 10^{-8} (\Omega_B M \epsilon)^{2/5} \eta^{-11/15} \quad (8)$$

The present temperature associated with this radiation, as $z_* \sim 10^2 \eta^{-2/3}$, is $E \sim E_{\max} z_*^{-1} \sim 10^2 \eta^{5/3}$ eV. Although equations (7) and (8) were derived previously¹², the 'energy' parameter η was there assumed to be arbitrary and epoch-independent, whereas, for any particular model of black hole accretion, the value of η may itself be determined by M and \dot{M} . In particular, for some disk accretion models¹⁴, there will be a band in the (\dot{M}, M) plane in which one would expect to produce optically thin bremsstrahlung radiation with a well-defined temperature. From the Bondi accretion formula and equation (4), the accretion rate at the epoch z_* is

$$\dot{M} \approx 10^{13} \Omega_B^{-3/5} M^{7/5} \epsilon^{2/5} \eta_*^{-12/15} \text{ g s}^{-1} \quad (9)$$

and the associated 'disk' temperature¹⁴ is

$$\theta_* \equiv 10^8 \eta_* \approx 10 \left(\frac{M}{M_\odot} \right)^{1/2} \text{ K} \approx 10^{7.5} \Omega_B^{-3/10} (M \epsilon)^{1/5} \eta_*^{-6/5} \text{ K} \quad (10)$$

where η_* specifies the value of η at z_* . One expects an optically thin bremsstrahlung spectrum providing θ_* is in the range 10^7 – 10^9 K.

We will not attempt here to investigate whether material accreted pregalactically would, in fact, form an accretion disk; this involves issues too complicated to discuss in the present context. However, note that the present hypothesis would almost certainly fail if the black holes did not form accretion disks. For example, were the accretion spherically symmetric, radiation could still be generated with an appreciable efficiency due to magneto-hydrodynamic dissipation¹⁵. However, because the dominant radiation mechanism would be synchrotron losses in this situation, one could not expect to produce optically thin bremsstrahlung radiation.

Equations (8) and (10) determine Ω_x and the present background temperature T_x in terms of Ω_B and M :

$$\Omega_x \approx 10^{-8} \Omega_B^{1/2} (M \epsilon)^{1/3}, \quad T_x \approx \theta_* z_*^{-1} \approx 10^6 \Omega_B^{-5/22} (M \epsilon)^{5/33} \text{ K} \quad (11)$$

Thus the temperature and (to a lesser extent) the density of the background are remarkably insensitive to the values of Ω_B and M . Indeed, for most hypotheses of pregalactic black hole formation, one would expect to produce a background of optically thin bremsstrahlung X rays, providing the holes accrete according to the disk mode. On the other hand, because the observed value for T_x is specified so precisely, one can still infer the necessary values of M and Ω_B to within an order of magnitude. The present temperature of the radiation, $T_x \sim 10^2 \eta_*^{5/3}$ eV, is 45 ± 5 keV (as required¹) providing $\eta_* = 40 \pm 3$, that is providing the accretion at z_* produces radiation with

energy $E_{\max} \approx 0.4$ MeV. In this case $z_* \sim 10$ and, because $\Omega_x \sim 10^{-8}$, equation (11) implies

$$\Omega_B \sim 10^{-6}, \quad M \epsilon \sim 10^8 \quad (12)$$

Values for Ω_B and $M \epsilon$ much different from these would fail to produce either the density or the temperature of the X-ray background. The largest reasonable value for ϵ is about 0.1; in this case one needs $M \sim 10^9$. Note that the radiation produced at pregalactic epochs before and after z_* will also contribute to the X-ray background at energies around kT_x . However, these contributions will be dominated by the radiation produced at z_* itself if the holes are then producing optically thin bremsstrahlung radiation.

In the above discussion we have assumed that the black holes have a unique mass, whereas one might expect them to span a range of masses. However, equation (11) shows that the dominant effect on Ω_x and T_x comes from the value of M which maximises the products $\Omega_B M^{2/3}$ and $\Omega_B^{-1} M^{2/3}$, respectively. For the anticipated¹⁰ types of mass spectra, the largest holes would have the biggest effect on both Ω_x and T_x , although they may not dominate the total black hole density. The largest holes would also determine the value of T_{\max} . With the favoured parameters ($\Omega_B \sim 10^{-6}$, $\epsilon M \sim 10^8$, $\eta_* \sim 40$), equation (4) implies that the pregalactic medium would have attained a maximum temperature $T_{\max} \sim 10^5$ K at a redshift of ~ 10 . The temperature of the intergalactic medium today would be somewhat different from this (depending on which postgalactic heating sources operate) and its density would depend on how much gas was left over after galaxy formation. It is interesting that recent estimates¹⁶ of the density and temperature of the intergalactic medium inferred from observations of Ly α absorption lines in quasars indicate that T was indeed about 10^5 K at $z \sim 2$.

Note that one cannot expect this picture to produce an exactly 45-keV bremsstrahlung spectrum, partly because the radiation comes from a range of redshifts and perhaps a range of black hole masses, but also because even an individual hole will not have a unique temperature. There will be a superposition of spectra from different points on the accretion disk. All one can state is that the dominant contribution may fit a 45-keV spectrum; a more detailed analysis would be needed to determine whether the distortion due to the lesser contributions would be within the error bars of the data. The important point is that the background conditions should be very uniform before galaxy formation, so one would not expect the range of spectra exhibited in postgalactic sources like quasars. Note that Barrow and Silk have also suggested that pregalactic black hole accretion could generate the X-ray background¹⁹. However, they do not attempt to fit the actual spectrum; nor do they allow for the effect of the accretion on the thermal history of the Universe.

Finally, we consider the radiation produced by the black holes during the present epoch. The production by holes which reside in galactic haloes or clusters has been estimated previously¹². Such holes might in principle generate the density of the background radiation, for suitable values of Ω_B and M , but one would not expect them to accrete at a rate sufficient to produce a 45-keV optically thin bremsstrahlung spectrum. For example, if one assumes reasonable values for n and T ($n \sim 10^{-3}$, $T \sim 10^6$ in haloes; $n \sim 10^{-3}$, $T \sim 10^8$ in clusters), then equation (10) requires $M \sim 10^9 M_\odot$ for halo holes and $M \sim 10^{14} M_\odot$ for cluster holes. Both these possibilities are precluded by observation¹⁰. Liang has considered the possibility that the holes are intergalactic¹⁷ and that the ambient conditions are there as inferred by Sargent *et al.*¹⁶. He argues that such holes could also produce the 45-keV background density. On the other hand, with the sort of values for Ω_B and M required, it is perhaps more plausible to envisage the holes—rather than inhabiting any of the environments discussed above—presently residing in galactic nuclei. For a large black hole would naturally act as a condensation nucleus for a galaxy¹⁸ and the sorts of holes which have been hypothesised to power quasars and Seyfert galaxies¹¹

would indeed have values for Ω_B and M of the order of those we have inferred. In this case one could identify the sources of the hard X-ray background as the precursors of the most prominent X-ray sources at the present epoch. This would please proponents of the view that known sources can produce the background if one allows for evolutionary effects.

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Possible transfer of lunar matter to Earth due to a nearby supernova

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Theories of supernova explosion (SNEs)¹⁻³ as well as correlation of NO_3^- in Antarctic ice cores with the dates of historical supernovae⁴, suggest that many Type I SNE may yield an intense 10^{50} erg emission of γ rays. Such a SNE may be expected to occur within a few tens of light years of our Solar System once every hundred million years or so^{5,6}. A short γ -ray pulse of this magnitude would not only directly affect the terrestrial atmosphere in ways that can be important for surface life⁵, but also be absorbed by the lunar surface. Depending on the γ -ray energies involved, the upper centimetre of lunar surface soil can be evaporated by heating or ejected by the emergence at the surface of a rising pressure pulse. Such expelled lunar soil, moving at speeds near the $2 \times 10^5 \text{ cm s}^{-1}$ lunar escape velocity, could be captured by the Earth's gravitational field and gradually settle through the atmosphere to the Earth's surface. Geological strata for that epoch might then be expected to show an overabundance of iridium (but probably less than that found by Alvarez *et al.*⁷ in 65-Myr-old sedimentary layers at Gubbio). This would occur since the expelled lunar surface soil should be characterised by an Ir abundance greatly enriched, relative to terrestrial of lunar rocks, by micrometeorite bombardment.

SNE models often involve a configuration in which the dense exploding stellar core is surrounded by a rather minimal atmosphere^{3,5}. Under such circumstances, the outgoing supernova shock wave propagating through a very steep density gradient will become extremely relativistic. The hot relativistically expanding matter at the shock front emits a burst of radiation when only one optical depth of matter remains to be penetrated. The interval over which this radiation pulse arrives is greatly compressed, due to the relativistic expansion of the radiating matter. Colgate originally estimated a γ -ray flash (with a total energy $\sim 10^{48}$ erg) compressed into a pulse of duration $\leq 10^{-5}$ s (refs 1, 5). There exists a great variation in estimated γ -ray energies, from \sim few MeV upward, and in total energies of the pulse up to $\sim 10^{50}$ erg (refs 2, 3).

A recent analysis of NO_3^- concentrations in an Antarctic ice core revealed the presence of four spikes coincident with historically known supernova⁴. Two of these are known to have been Type I supernovae; the other two are not identified. To explain the large magnitudes of the four NO_3^- spikes, it seemed that at least 10^{50} ergs of X rays or γ rays would have to be emitted by each SNE. This radiation could, however, have arrived over an interval as long as a year rather than as a flash.

No γ -ray flashes have yet been detected in coincidence with extragalactic SNEs. However, the γ -ray flash, which should arrive well before the SN is visually observed, may well be associated only with a specific subclass of Type I SNEs. Furthermore, if the characteristic energies of the γ -rays are very high, it is possible these bursts would have escaped detection: fairly massive detectors need to be flown in satellites to allow the full e^+ shower to develop. We note also that γ rays above $\sim 10^{15}$ eV will produce e^+ pairs in collisions with the universal 3 K black-body radiation photons. This limits the detectability of such γ -ray sources to those within about 10^4 light yr. γ -ray flashes from the historical galactic supernovae could then have reached us, but not those from explosions in other galaxies.

We shall next consider some consequences of an assumed 10^{50} erg γ -ray flash from a SNE at a distance of 30 light yr or less from our Solar System. This would give an incident γ -ray flux in excess of $\sim 10^{10} \text{ erg cm}^{-2}$ locally.

The Earth's atmosphere shields its surface from the direct impact of an intense X-ray or γ -ray pulse. Effects on the surface would be indirect, such as those resulting from NO production and subsequent O_3 destruction. In contrast, the lunar surface receives the full flux. The detailed consequences of this exposure depend somewhat on the regime of incident γ -ray energies.

MeV γ rays, for which Compton scattering is the main energy deposition mechanism, are absorbed by $\sim 1 \text{ g cm}^{-2}$ of lunar surface matter. Thus, $\sim 10^{10} \text{ erg g}^{-1}$ of rapidly absorbed energy will be deposited in the solid surface layer, which has a density of $\sim 1 \text{ g cm}^{-3}$, down to a depth of 1 cm. This will increase the temperature of the centimetre layer to 10^3 – 10^4 K and generally vaporise it, assuming the γ -ray energy is deposited in less than a second (for $T \sim 3 \times 10^3$ K). The kinetic energy required for escape from an isolated moon is also $\sim 10^{10} \text{ erg g}^{-1}$, roughly comparable to the energy provided by the absorbed γ -ray flash. (Because of the Earth's gravitational pull, an additional 40% is needed to escape the Earth-Moon system.) Therefore, the hot evaporated vapour, which temporarily provides the Moon with an atmosphere of density $\sim 10^{-12} \text{ g cm}^{-3}$, will expand to fill and ultimately overflow the Roche lobe. A substantial fraction of this material, flowing through the inner lagrangian point into the Earth's Roche lobe, should ultimately reach the Earth. It would initially form a ring, at a radius of the order of 10^{10} cm , in which the particles have roughly keplerian velocities. Because of the extremely low gas density, $\sim 10^{15} \text{ g cm}^{-3}$, the mean free path for molecular collisions is about 10^{10} cm , that is comparable to the ring dimension. This causes the gas to behave as an extremely viscous fluid. The keplerian differential rotation then causes the main part of the disk to contract on a rapid time scale, if the outer material can be dispersed. During the descent of this lunar matter to the Earth, it would be affected by the solar wind; however, the wind pressure $\sim 10^{-10} \text{ dyn cm}^{-2}$ on 10^{18} g of falling vapour is insufficient to sweep even fully ionised matter of this kind away from the Earth is less than 10 yr (M. Rees, personal communication). Neutral gas will survive for much longer. The accreted lunar gas may take several decades to move (mainly by advection) down through the upper atmosphere and stratosphere and ultimately reach the Earth's surface.

A somewhat different theory for an ejection of lunar surface matter would be required if the SNE γ -ray flash were characterised by photon energies $\gg 100 \text{ MeV}$, arriving within an interval of the order of 10^{-5} s. For these conditions, the incident γ -ray energy is deposited at a depth of a few tens of cm, mainly by ionisation resulting from an electron-position shower which develops over several radiation lengths (l). If the duration of the γ -ray pulse is $\leq 10^{-4}$ s (l over the local sound speed), the energy

is deposited on a time scale less than that for thermal expansion in response to the initial differential heating. The resulting initial pressure excess, the product of the temperature rise, the coefficient of thermal expansion, and the volume compression modulus, is of the order of $10^{10} \text{ l}^{-1} \sim 10^9 \text{ dyn cm}^{-2}$ within the main energy deposition layer. Emergence of this pressure impulse at the lunar surface which has weak or negligible tensile strength should result in mass ejection. Matter ejected at the speed of sound in lunar matter has about the 10^5 cm s^{-1} speed needed to escape from the Moon and still be captured by the Earth.

If a SNE γ -ray flash and the consequent transfer of lunar material to the Earth occurred >60 Myr ago, it would be difficult to find evidence of it on the surfaces of lunar rocks today. Micrometeorite bombardment erodes those surfaces at the rate of $10^{-1} \text{ cm Myr}^{-1}$ (ref. 8), so that direct evidence for or against melting and recrystallisation or fragmentation of the outer centimetre or so of lunar surface matter is unlikely (G. Turner, personal communication). (Some evidence for such a flash might still be found in the form of glassy fragments, which were buried before they could be eroded.) The lunar samples studied have also come from a relatively small region, about 10^3 km across, which would have one chance in two of having been on the dark side of the flash.

If a strong $10^{10} \text{ erg cm}^{-2}$ γ -ray flash is likely every 10^8 years from SNEs uniformly distributed in space and time, a flash of strength $10^{10} \text{ erg cm}^{-2}$ would be expected to have occurred as recently as 10^8 yr ago. Thus, about 10^6 years ago there might have been a final γ -flash of intensity $5 \times 10^8 \text{ erg cm}^{-2}$. This would not have been intense enough to evaporate lunar surface matter but could have melted almost 1 g cm^{-2} of it; that is, to a depth of a few millimetres if the flash γ rays had energies in the X-ray to MeV region. Erosion would not yet have removed all of the melted-recrystallised surfaces. One is tempted to speculate that such a flash might still be in fact observable in the form of the glassy patches emphasised by Gold⁹, who proposed a sudden (solar) flash for the original melting. On the other hand, a relatively nearby SNE may be 'common' only when the Solar System is within a spiral arm, for example, of the order of 60 Myr ago. A SNE γ -ray flash giving $5 \times 10^8 \text{ erg cm}^{-2}$ some 10^6 years ago would then be less likely.

A possible consequence of the transfer of $\sim 1 \text{ g cm}^{-2}$ of the lunar surface to that of the Earth rapidly enough to be, in effect, instantaneous over geologically measurable intervals, is element abundance anomalies at appropriate places in the terrestrial sedimentary layers. Alvarez *et al.*⁷ report a large enhancement in the abundance of Ir, from $0.25 \times 10^9 \text{ g per g}$ of limestone to $6.35 \times 10^{-9} \text{ g per g}$ in a 1-cm layer at Gubbio. This anomaly is identified as occurring at the Cretaceous-Tertiary boundary about 65 Myr ago. The isotope abundance ratio remained normal.

The Earth's surface is deficient in Ir relative to the meteorites by a factor of more than 10^3 . While lunar rocks are also characterised by a large deficiency, the upper centimetre of lunar soil, which is strongly adulterated by micro-meteorite bombardment, can be expected to show significant Ir concentrations. Analyses of Apollo 11 and Apollo 12 lunar soil samples^{10,11} reveal Ir abundances up to $\sim 10 \times 10^{-9} \text{ g per g}$; this represents typically an average over the upper few metres or so of the regolith. After a study of the overall character of the trace-element abundance patterns, this enrichment has been attributed^{10,11} to the addition of carbonaceous chondrite-like material at a rate of the order of $4 \times 10^{-9} \text{ g cm}^{-2} \text{ yr}^{-1}$ over $4.5 \times 10^9 \text{ yr}$. (The Ir concentration in chondrites is over 1,000 times that found in the crust of the Earth and 100 times that of lunar soil.) At this rate, 0.4 g cm^{-2} of the meteoritic material would be accumulated in the 10^8 year intervals between our hypothesised occurrences of intense γ -ray flashes. The rapid transfer of this amount of meteoritic matter alone to the surface of the Earth would provide 0.03 g cm^{-2} which, when mixed into 1 g cm^{-2} of terrestrial soil, would yield an Ir concentration of about $15 \times 10^{-9} \text{ g per g}$. However, turnover of lunar surface

matter occurs at a rate given approximately by 0.1 cm Myr^{-1} (ref. 8). If there were mixing to a depth of several centimetres over a 10^8 year interval, the average Ir content of this surface material would be of the order of $3 \times 10^{-8} \text{ g per g}$. There is no compelling reasons for the lunar surface to be homogeneous: some patches may have more than this while most probably have less. The Ir results obtained by Alvarez *et al.*⁷ might be understood in this way only if the top several g cm^{-2} of lunar soil were evaporated and now contributed the measured Ir at the appropriate geological stratum. Alternatively, we might assume the top g cm^{-2} has a significantly higher average value, $\sim 10^{-7} \text{ g per g}$, since there might be large patches which do not undergo such thorough mixing before evaporation. If most of this material reached the Earth and mixed with 1 g cm^{-2} , the Ir discontinuity obtained at Gubbio could again follow, but its magnitude does seem much larger than is easily explained by lunar surface matter transfer.

Orbits of near Earth micrometeoritic particles will be strongly altered by non-symmetric surface evaporation resulting from a γ -ray flash. This too may cause a temporary increase in the fall of very Ir rich matter onto the Earth (A. Fabian, personal communication).

A $10^{10} \text{ erg cm}^{-2}$ flash would lead directly to very strong ozone suppression in the Earth's atmosphere, and thus very greatly increased solar UV radiation at the Earth's surface for about a decade⁵. In addition, during the long period of lunar soil mass transfer through our atmosphere, the transparency of the dirtied terrestrial atmosphere to visible and Ir radiation would be very greatly altered, even if the lunar matter remained mainly gaseous. The added lunar matter could be almost a tenth as abundant as atmospheric CO_2 . If the lunar matter were dustlike, the direct atmospheric albedo together with changed cloud seeding would significantly influence the surface climate.

The detailed consequences of these various possible environmental modifications on biological species are not known. It seems reasonable to speculate that a biological cataclysm may have resulted. A key question is whether or not the great extinctions at the end of the Cretaceous age in which approximately one-quarter of all species, including the dinosaurs, died were sufficiently simultaneous¹². Some recent data incorporating times of magnetic field reversals have been interpreted as indicating that the faunal overturn at Gubbio took $<10^4 \text{ yr}$ (ref. 13) and that the Foraminifera and dinosaur extinctions in Alberta were 'simultaneous' with a maximum error of 10^5 yr (ref. 14). While such data hardly insist on a sudden catastrophic explanation, they do question the notion that there are no strong correlations among the extinctions or that they are all caused by slowly changing geological conditions.

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Reactions of OH radicals with gaseous sulphur compounds

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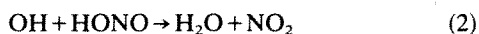
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Several recent papers¹⁻³ have discussed the origin of the sulphate aerosol in the stratosphere in terms of atmospheric carbonyl sulphide (OCS) and carbon disulphide (CS₂). There is, however, considerable uncertainty in the life cycles and budgets of these compounds. This arises from a lack of knowledge of the various possible sources and also from the differences in the reported rate constants for the reactions of OCS and CS₂ with hydroxyl (OH) radicals^{4,5}, which provide a potentially large sink for these molecules in the troposphere. We report here measurements of rate constants and product formation for OH reaction with several sulphur compounds, including OCS and CS₂. The results support the suggestion that oxidation of CS₂ is a significant source of atmospheric OCS, but do not give conclusive evidence for a large sink for OCS in the troposphere.

Interest in atmospheric sulphur chemistry has recently focused on the sulphate component in the stratospheric aerosol layer which plays a significant role in the Earth's radiation budget. In addition to direct injection of SO₂ into the stratosphere from volcanic activity, an important role for OCS as a carrier of sulphur from the troposphere to the stratosphere has been suggested¹. Recent measurements⁶⁻⁹ show that OCS is indeed widely distributed in the atmosphere. Photolysis of OCS in the stratosphere releases atomic S which is oxidised and subsequently converted to H₂SO₄ aerosol. It has also been proposed that reaction of OCS with OH is the origin of the SO₂ present in the background troposphere¹⁰ and that a similar reaction of CS₂ is a source of atmospheric OCS as well as SO₂ (ref. 2).

In the present work rate constants were measured using a competitive rate technique, in pseudo-atmospheric conditions. Hydroxyl radicals were generated by the photolysis at 350 nm of ~5 p.p.m. of nitrous acid (HONO) in synthetic air at 1 atm pressure and ambient temperature (297 ± 2K), contained in a 200 l bag fabricated of Tedlar film. Photolysis of the mixture results in a steady state concentration of OH radicals which at small conversion is governed by the reactions (1) and (2)¹¹



[OH] can be conveniently monitored in this system by addition of a few p.p.m. of ethylene (C₂H₄) which, following attack by OH, is oxidised to two molecules of formaldehyde by a chain reaction in which OH is regenerated^{12,13}. By monitoring the rate of C₂H₄ decay, [OH] can be calculated from the relationship

$$-\frac{d \ln [\text{C}_2\text{H}_4]}{dt} = k_3 [\text{OH}] \quad (3)$$

k_3 is the rate constant for the reaction of OH + C₂H₄ which at atmospheric pressure has a value of $8 \times 10^{-12} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$

(ref. 14). Rate constants for the reaction of OH with the sulphur compounds were obtained relative to k_3 , by measurement of the decay of the sulphur compound and of C₂H₄ during photolysis in the HONO-Air mixtures for ~30 min. Expressing the integrated forms of equation (3) as ratios for the two substrates gives

$$\ln \frac{[\text{S}]_0}{[\text{S}]_t} = \frac{k_s}{k_3} \ln \frac{[\text{C}_2\text{H}_4]_0}{[\text{C}_2\text{H}_4]_t} \quad (4)$$

where the subscripts refer to concentrations at time 0 and t ; k_s is the rate constant for reaction of OH with the sulphur compound, S.

Ethylene, methyl mercaptan (MM), dimethylsulphide (DMS), dimethyldisulphide (DMDS) were all measured by gas chromatography using flame ionisation detection. H₂S, SO₂, OCS and CS₂ were measured using a flame photometric detector after separation on a 1 m Chromasil 316 column mounted in an all-PTFE chromatographic system. HONO, NO and NO₂ were measured using a commercial chemiluminescence detector. All gases and vapours were used as supplied commercially except for OCS from which H₂S impurity was removed to a level of <0.01% by passage through aqueous Cu(NO₃)₂.

Figure 1 shows a plot of the concentration-time data according to equation (4). Good straight line plots were obtained in the main, indicating that the removal of both substrates was dominated by reaction with OH. For CS₂ and DMS there was a tendency for their removal rates to increase relative to C₂H₄ later in the reaction. This can be attributed in part to reaction with oxygen atoms, which are produced from photolysis of NO₂ formed during photolysis. Also CS₂ absorbs light in the 300–350 nm region; in the absence of HONO, the decay rate of CS₂ was a factor of 3 slower indicating that although direct photolysis was significant in the HONO-CS₂ system, it was not the major decay route for CS₂.

Table 1 Rate constants for OH + sulphur compounds

Gas	$\frac{k_s}{k_{\text{C}_2\text{H}_4}}$	$k_s (\text{cm}^3 \text{ molecule}^{-1} \text{ s}^{-1} \times 10^{-12})^*$
DMDS	28 ± 10	223 ± 80
MM	11.3 ± 1.1	90.4 ± 8.5
DMS	1.14 ± 0.18	9.1 ± 1.4
H ₂ S	0.62 ± 0.04	5.0 ± 0.3
SO ₂	0.09 ± 0.02	0.72 ± 0.16
CS ₂	0.06 ± 0.02	0.43 ± 0.16
OCS	≤ 0.005	≤ 4 × 10 ⁻²

* Using $k_{\text{C}_2\text{H}_4} = 8 \times 10^{-12} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$.

Table 1 shows a summary of the rate constant data from the plots according to equation (4). The OH rate constants are in good agreement with previous results for SO₂ (at 1 atm pressure)¹⁵, H₂S (refs 15, 16) and DMS (ref. 14). A value for k (OH + DMDS) has not apparently been reported before. For both CS₂ and MM the values are both higher than measured directly using the resonance fluorescence technique at low total pressures^{4,5,14}. We conclude that the reaction of OH with MM is enhanced at high pressure. The position for CS₂ is less well defined; the CS₂ value given has been corrected for the apparent contribution of ~40% to the CS₂ decay, due to photolysis and O(³P) reaction, but the possibility of a systematic error in this determination cannot be entirely ruled out. We are, therefore, unable to conclude definitely whether there is a pressure effect on $k(\text{OH} + \text{CS}_2)$ but our results are certainly more consistent with the value reported by Kurylo⁵ that is, $1.8 \times 10^{-13} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ than with the upper limit value of Atkinson *et al.*⁴, $7 \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$.

For OCS, the absence of any measurable decay gave an upper limit comparable with the largest reported value for $k(\text{OH} +$

OCS) (ref. 5), that is, $5.66 \times 10^{-14} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$. However, observation of the effect of a large excess of OCS on the apparent $[\text{OH}]$ calculated from equation (3) in the $\text{HONO}-\text{C}_2\text{H}_4$ system, indicated that the true value of $k(\text{OH} + \text{OCS})$ may be considerably lower than this upper limit. A value of $\sim 8 \times 10^{-15} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ could be derived from the data but uncertainties in the detailed chemistry precluded an accurate determination of this rate constant.

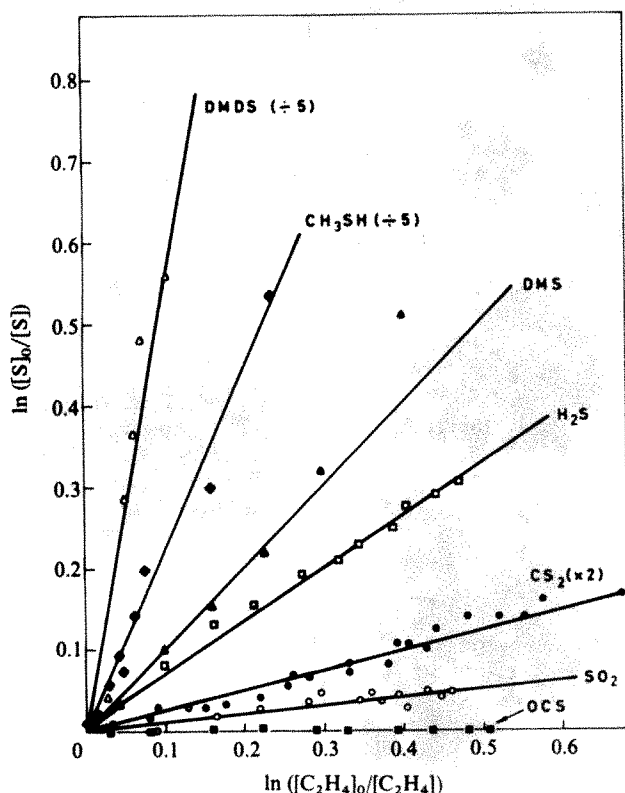
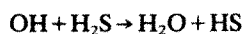
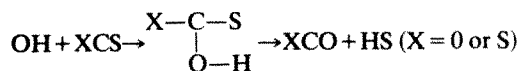


Fig. 1 Plot of $\ln [S]_0/[S]$, versus $\ln [C_2H_4]_0/[C_2H_4]$, for the decay of sulphur compounds and ethylene during photolysis in HONO/air mixtures at atmospheric pressure and 297 K.

Investigation of product formation in these systems revealed that SO_2 is a major product in the reaction of OH with all the S compounds investigated, with the exception of SO_2 itself which is oxidised to an H_2SO_4 aerosol¹⁷. H_2S was converted quantitatively to SO_2 and, as the initial attack of OH with H_2S undisputably occurs through the reaction



we conclude that HS is efficiently converted to SO_2 in our experimental conditions. The observation of SO_2 formation in the $\text{OH} + \text{CS}_2$ and $\text{OH} + \text{OCS}$ system therefore supports the suggestion⁵ that these reactions proceed through an addition complex, which yields HS as a primary product:



Further indication that this is a major pathway for CS_2 comes from the observation of the production of OCS with a high yield ($\sim 100\%$) in the $\text{OH} + \text{CS}_2$ system.

Note the significance of the results in the context of the behaviour of CS_2 , OCS and the organic sulphides in the global

sulphur cycle. The ubiquitous presence of OH in the troposphere provides a sink mechanism for gases with which it reacts. A relationship between tropospheric lifetime and OH reaction rates has been computed using a two-dimensional model¹⁸; our value of $k(\text{OH} + \text{CS}_2)$, that is, $4.3 \times 10^{-13} \text{ cm}^3 \text{ molecule}^{-1} \text{ s}^{-1}$ gives a lifetime of 0.2 yr for CS_2 .

Our results show that the reaction of CS_2 with OH radicals provides a potentially large source of OCS in the troposphere. The major uncertainty in the calculation of the source strength is due to the paucity of observational data for atmospheric CS_2 . The only reported measurements of CS_2 in air⁷, gave concentrations in the range 0.07–0.37 p.p.b. (parts per 10⁹). Subsequent measurements have confirmed that CS_2 concentrations near the lower end of this range are normally present in the atmosphere at Harwell (S. A. Penkett, personal communication). If the value of 0.07 p.p.b. is taken to be representative of background tropospheric air, the source strength of OCS corresponding to a lifetime of 0.2 yr is $2 \times 10^{12} \text{ g(S) yr}^{-1}$. This is considerably larger than estimates of OCS emissions from identified sources, that is, biomass burning, $2.4 \times 10^{11} \text{ g(S) yr}^{-1}$ (ref. 19), soil emissions $2.3 \times 10^{11} \text{ g(S) yr}^{-1}$ (based on flux measurements in the US²⁰).

The source of OCS from CS_2 alone is considerably larger than estimates of the amount of OCS removed by photolysis in the stratosphere^{1,2}, $(2-5) \times 10^{10} \text{ g(S) yr}^{-1}$, and implies a large sink for OCS in the troposphere. Measurements⁶⁻⁹ of tropospheric OCS have shown that it is fairly uniformly distributed with a concentration of about 0.4 ± 0.1 p.p.b., and, to balance the source from CS_2 , would require a lifetime of 1.1 yr for OCS. A sink of this magnitude could be provided by reaction with OH only if the 'high' value for the rate constant $k(\text{OH} + \text{OCS})$ (ref. 5) was correct. However, the true value may be lower by up to a factor of 10, which suggests that an additional sink for OCS may be operative. Physical removal of OCS on vegetation²¹ or at ocean surfaces may fulfil this role.

The reaction of OH with CS_2 as formulated above also provides a source of SO_2 , equivalent in size to that of OCS. In this case, however, the source is small compared with the global flux of sulphur in the troposphere $1 \times 10^{14} \text{ g(S) yr}^{-1}$ (ref. 22) of which about 60% is due to SO_2 emission from fossil fuel combustion. The remainder of the tropospheric sulphur is thought to originate from oxidation of H_2S , DMS and other organic sulphur compounds, primarily of natural origin. The present work confirms their short tropospheric lifetime due to rapid reaction with OH, and also shows that SO_2 is an important product of these reactions in pseudo-atmospheric conditions.

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Enrichment of ^{210}Pb and ^{210}Po in the sea-surface microlayer

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Chemical fractionation at the air-sea interface is frequently suggested¹⁻⁵ as a possible mechanism contributing to the enrichment of a number of trace metals in the marine atmosphere^{6,7}. The origin of the long-lived radon daughters (^{210}Pb , ^{210}Bi and ^{210}Po) in the atmosphere is of special interest because of their use in the estimation of tropospheric aerosol residence times⁸. It has been proposed⁹ that injection of a fractionated marine component by bubbles bursting at the sea surface might be responsible for the occurrence of anomalously high atmospheric $^{210}\text{Po}/^{210}\text{Pb}$ ratios in Antarctica¹⁰ and Hawaii¹¹. Here we report the first measurements of ^{210}Pb ($t_{1/2} = 22.3$ yr) and ^{210}Po ($t_{1/2} = 138$ d) in the sea-surface microlayer. These measurements were made to determine whether or not a significant flux of these radionuclides from the sea surface to the atmosphere could occur. We also introduce a method for treating chemical data that we believe may be useful in determining the origin of the trace-metal enrichments often observed^{1,2} in the microlayer.

The sea-surface microlayer is the uppermost thin skin of the oceanic water column. It is often characterised by anomalously high concentrations of heavy metals and other trace substances thought to be associated with particulate matter and surface-active organic material^{1,2}. The thickness of the microlayer is defined by the method used to sample it. We used a plastic screen in a modification of the technique of Garrett which samples the upper 300 ± 50 μm (refs 12, 13). Because this was an initial attempt to detect enrichments of ^{210}Pb and ^{210}Po in the microlayer, we deliberately chose to collect our screen samples in calm sea conditions when visible surface slicks were present. Bulk surface-water samples were collected by immersing polyethylene jugs to a depth of about 20 cm and allowing them to fill. We also analysed a single sample of foam collected along the shore of a coastal pond. This material is believed to represent a more concentrated example of the anomalously enriched layer than samples provided by the screen technique^{14,15}. Details of the analytical procedures are given elsewhere¹⁶.

Results are shown in Table 1. In all cases both ^{210}Pb and ^{210}Po showed enrichments in the sea-surface microlayer samples in comparison with bulk surface-water samples. The degree of enrichment seemed to correlate with film pressure (depression of surface tension) estimated by the oil-drop method^{13,17}, although film pressures were always near the limit of detection. The greatest enrichments occurred in the foam sample from Oyster Pond. Thus a correlation exists between enrichments of the radionuclides and the presence of surface-active material.

To assess the role of the sea surface in supplying metal-rich aerosols, one must determine whether the enrichments in the microlayer are maintained by deposition of atmospheric material or by concentration of the metals from seawater. Only in the latter case can the sea act as a source. The $^{210}\text{Po}/^{210}\text{Pb}$ activity ratio may provide an important clue. Table 2 lists representative values of the $^{210}\text{Po}/^{210}\text{Pb}$ ratio in atmospheric fallout and in seawater and marine particulate matter from both coastal and open-ocean environments. The atmospheric ratio is much lower than the ratios in bulk surface seawater measured in this study, yet the $^{210}\text{Po}/^{210}\text{Pb}$ ratios in the microlayer samples show only small departures from the bulk seawater values. This comparison suggests that the ocean may be an important source of the enrichments found in the microlayer.

A more quantitative treatment of this question can be made by consideration of a simple model in which the surface-microlayer enrichment consists of two components: an atmospheric component and an oceanic component supplied by concentration from seawater and upward transport to the air-water interface. This latter supply process may involve chemical fractionation. It is assumed that processes removing material from the microlayer, either physical injection into the atmosphere or mixing downward in the water column, involve no chemical fractionation. With this assumption we can write for ^{210}Pb :

$$\Delta_{\text{Pb}} = \text{Pb}_m - \text{Pb}_b = \varepsilon_{\text{Pb}} \text{Pb}_b + \text{Pb}_a \quad (1)$$

where the ^{210}Pb enrichment, Δ_{Pb} , is the difference between the ^{210}Pb concentration in the microlayer, Pb_m , and the concentration in bulk surface seawater, Pb_b . This concentration difference consists of an enriched oceanic component, $\varepsilon_{\text{Pb}} \text{Pb}_b$, and an atmospheric component, Pb_a . A similar equation can be written for ^{210}Po :

$$\Delta_{\text{Po}} = \text{Po}_m - \text{Po}_b = \varepsilon_{\text{Po}} \text{Po}_b + \text{Po}_a \quad (2)$$

Finally we assume that the atmospheric $^{210}\text{Po}/^{210}\text{Pb}$ ratio, R_a , is known:

$$R_a = \text{Po}_a / \text{Pb}_a \quad (3)$$

Equations (1)–(3) contain four unknown quantities, so it is not possible to solve them uniquely. It is possible, however, to derive a relationship between two important quantities. Let α denote the fractionation between ^{210}Pb and ^{210}Po during their transport from bulk seawater to the microlayer. This fractionation is given by

$$\alpha = \varepsilon_{\text{Po}} / \varepsilon_{\text{Pb}} \quad (4)$$

so if $\alpha > 1$, then ^{210}Po is preferentially (relative to ^{210}Pb) transported to the microlayer. This condition is probably necessary if the sea surface is to act as a source for the anomalously high $^{210}\text{Po}/^{210}\text{Pb}$ ratios in Antarctic aerosols¹⁰, because $^{210}\text{Po}/^{210}\text{Pb} < 1$ in most ocean surface water. (Coastal waters in Vineyard Sound (Table 1) and other locations^{18,19} are often the exception with $^{210}\text{Po}/^{210}\text{Pb} > 1$. The origin of the unsupported ^{210}Po in these waters is not understood. Over most of the ocean surface,

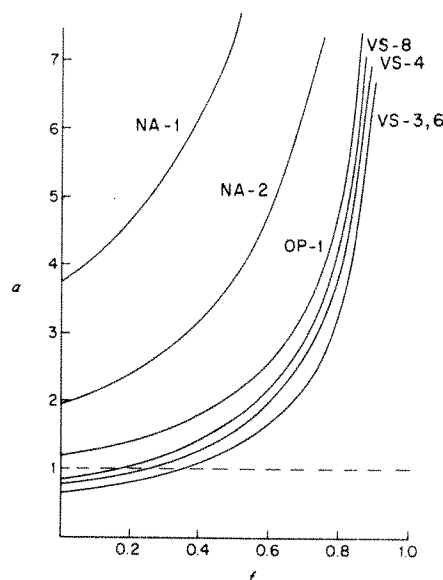


Fig. 1 Results of the model calculations for $R_a = 0.1$. Each screen sample is represented by a curve. In those cases for which more than one bulk surface-water sample was collected on the same date and in the same location, the results from Table 1 were averaged. For $\alpha = 1$ there is no chemical fractionation. For $f = 1$ all of the enrichment of ^{210}Pb in the microlayer is contributed from the atmosphere. The curves are fairly insensitive to variations of R_a in the range 0–0.2.

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Table 1 Analytical results*

Sample	Type	Description of sampling conditions	Film pressure† (dyn cm ⁻¹)	²¹⁰ Pb (d.p.m. per 100 kg)	²¹⁰ Po	Activity ratio ²¹⁰ Po/ ²¹⁰ Pb	ΔPo/ΔPb
Vineyard Sound, Massachusetts (14 July 1977)							
VS-1	Bulk	—	—	3.5±0.1	9.6±0.4	2.8±0.2	—
VS-2	Bulk	—	—	1.8±0.1	6.8±0.3	3.8±0.3	—
VS-3	Screen	Visible surface slicks, deliberately sampled	>1	12.5±0.3	29.6±0.9	2.4±0.1	2.2
VS-4	Screen	Slicks present but avoided	~1	6.6±0.3	18.3±0.6	2.8±0.1	2.6
Vineyard Sound, Massachusetts (5 September 1977)							
VS-5	Bulk	—	—	5.0±0.2	7.0±0.4	1.41±0.09	—
VS-6	Screen	Visible slick, some quantities of foam sampled	>1	41.3±1.6	51.5±1.9	1.25±0.06	1.2
VS-7	Bulk	—	—	3.2±0.1	7.0±0.7	2.2±0.2	—
VS-8	Screen	Patches of slick present but avoided	<1	6.0±0.2	10.0±0.5	1.7±0.1	1.6
Oyster Pond, Falmouth, Massachusetts (29 September 1977)							
OP-1	Foam	—	—	1,360±30	430±50	0.31±0.04	0.32
OP-2	Bulk	—	—	36.8±1.0	9.6±0.7	0.26±0.02	—
North Atlantic Ocean 45°50'N, 64°10'W (16 September 1977)							
NA-1	Screen	Patches of slick present, sample gathered both in and out of slick	~1	16.4±0.3	15.0±0.7	0.91±0.05	2.6
NA-2	Screen	Patches of slick present, sample gathered both in and out of slick	~1	21.3±0.7	21.7±1.2	1.02±0.06	1.7
NA-3	Bulk	—	—	14.7±0.5	10.5±0.6	0.71±0.05	—

* Uncertainties listed are standard errors based on counting statistics.

† These are approximate values. All measurements were near the detection limit, and there was often some variation in film pressure during each sampling period because of slick patchiness.

however, $^{210}\text{Po}/^{210}\text{Pb} < 1$ because of biological uptake and transport of ^{210}Po to deeper water²⁰. We now let f denote the fraction of the microlayer ^{210}Pb enrichment that is contributed from the atmosphere, and we can write

$$f = \text{Pb}_a / (\text{Pb}_a + \text{Pb}_b) \quad (5)$$

With these definitions and with equations (1)–(3), we derive the following equation:

$$\alpha = (\Delta\text{Po}/\Delta\text{Pb} - fR_a) / (1 - f)R_b \quad (6)$$

where $R_b = \text{Po}_b/\text{Pb}_b$. Thus for any microlayer/bulk-seawater sample pair we can define a relationship between α and f . Note that this relationship is independent of the degree of dilution of the microlayer sample by bulk seawater.

Figure 1 shows curves based on equation (6) and the data in Table 1. Although our data do not allow a unique solution, the curves in Fig. 1 allow some limitations to be placed on their interpretation. We note that as f approaches a value of 1.0 (no oceanic contribution), α increases very rapidly. In other words, the more dominant the atmospheric contribution to the microlayer enrichment of ^{210}Pb becomes, the more strongly fractionated the oceanic contribution must be to compensate for the low $^{210}\text{Po}/^{210}\text{Pb}$ ratio in the atmospheric component. We believe that a reasonable interpretation of the data requires that at least part of the microlayer enrichments be supplied by concentration of ^{210}Pb and ^{210}Po from the bulk seawater.

At the other extreme, by setting $f = 0$ (no contribution from the atmosphere), we obtain the minimum possible value for α . To explain our open-ocean results (samples NA-1 and NA-2), we require $\alpha > 1$ no matter how small a value is assumed for f ; that is, concentration of ^{210}Pb and ^{210}Po from the bulk seawater must involve chemical fractionation such that ^{210}Po is preferentially transported to the interface. For the coastal water samples (sample OP-1 and the VS samples), $\alpha \approx 1$ (little or no fractionation) if no atmospheric contribution is assumed. This reflects the similarity of $^{210}\text{Po}/^{210}\text{Pb}$ ratios in microlayer and bulk surface-water samples at the coastal sampling sites. Only for $f \geq 0.4$ must significant fractionation be postulated.

Experiments in which columns of seawater are bubbled with air suggest that trace-metal enrichments in the microlayer may be maintained by transport of particulate matter to the interface⁵. If this were the predominant mechanism for ^{210}Pb and ^{210}Po , and if it acted indiscriminately for all types of particulate matter, then it would be possible to estimate α from the known distribution of ^{210}Pb and ^{210}Po between dissolved and particulate forms in surface seawater by using the expression

$$\alpha = (^{210}\text{Po}/^{210}\text{Pb})_{\text{particulate matter}} / (^{210}\text{Po}/^{210}\text{Pb})_{\text{bulk seawater}} \quad (7)$$

In open-ocean surface water^{21,22} this quantity is in the range 2.4–8.8 (median 6.7). Coastal surface waters (ref. 18 and unpublished data) are characterised by values closer to 1.0, probably because of the contribution of resuspended bottom sediment to the particulate matter. This difference may account for the segregation of our open-ocean samples from our coastal samples (Fig. 1), but further testing of this hypothesis is required.

We have shown that the sea-surface microlayer is enriched in ^{210}Pb and ^{210}Po . Our interpretation of the data suggests that a significant fraction of the enrichment is maintained by concentration from the bulk surface seawater. In the open ocean this concentration process results in preferential transport of ^{210}Po to the air–sea interface. In coastal environments there may be a

Table 2 Representative values of the $^{210}\text{Po}/^{210}\text{Pb}$ activity ratio in seawater, marine particulate matter and atmospheric fallout

Material	Median	Range	Refs
Surface North Atlantic			21, 22
Seawater	0.52	0.08–1.09	
Particulate matter	4.6	0.9–5.3	
Long Island Sound, US			18
Water (total)	0.89	0.80–1.44	
35 μm Seston	1.3	1.2–2.4	
333 μm Seston	12	1.4–62	
Atmospheric fallout	0.03	~0–0.16	18
New Haven, Connecticut			

lesser degree of chemical fractionation. Because coastal waters often have $^{210}\text{Po}/^{210}\text{Pb}$ activity ratios > 1 , the sea surface is, in either case, a potential source of aerosols with $^{210}\text{Po}/^{210}\text{Pb} > 1$ as suggested by Turekian *et al.*⁹. The natural flux of microlayer material from the sea to the atmosphere, however, is not known.

Note finally that our analytical treatment of the microlayer enrichments was limited because only two elements were used. Further analysis by a multi-element approach is suggested.

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Self-reversal of natural remanent magnetisation in the Olby-Laschamp lavas

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In 1967 Bonhommet and Babkine¹ reported reversed magnetisation directions in two lavas at Laschamp and Olby (Chaîne des Puys, Auvergne, France). They argued that the remanent magnetisation might have originated either from an episode of reversed polarity of the geomagnetic field within the Brunhes normal epoch or from a process of self-reversal² occurring in these lavas. I report here experimental evidence that the natural remanent magnetisation (NRM) of certain samples of the Olby flow undergoes complete self-reversal during thermal demagnetisation. Other Olby samples show partial self-reversal of NRM at room temperature.

Radiometric dating initially gave age limits to the extrusion of the Laschamp and Olby basalts between 8,000 yr and 20,000 yr BP (ref. 3) and since then the reversed volcanics have been generally accepted as a well documented case of a recent excursion of the Earth's magnetic field⁴, named the 'Laschamp geomagnetic polarity event'. Although Denham and Cox⁵ proved that it was highly probable that the geomagnetic field remained constantly normal on a global scale between 13,300 and 30,400 yr BP, many authors^{6–11} tried to correlate other events such as the Gothenburg⁶, Gulf of Mexico⁷, Maelifell¹⁰, Erieau¹¹ excursions with the Laschamp reversal. Age dating techniques have recently been refined. As a result of new K–Ar, $^{40}\text{Ar}/^{39}\text{Ar}$, $^{230}\text{Th}/^{238}\text{U}$ and thermoluminescence dates^{4,12–14} it

seems likely that the true age of the 'Laschamp event' is between 40,000 and 45,000 yr BP. Consequently magnetic correlation is now being made with somewhat older reported excursions, such as the Lake Mungo¹⁵ or Lake Biwa¹⁶ records.

Whatever the significance of these correlation attempts, the importance of geomagnetic events or excursions as magneto-stratigraphic marker horizons is unquestioned. Nevertheless, I would like to put forward an alternative explanation for the Laschamp event. Thermomagnetic experiments performed on the reversely magnetised Olby flow demonstrate that a self-reversal process acts on its NRM.

The occurrence of self-reversal in igneous rocks has long been debated. Extensive investigations have been postponed because of the overwhelming evidence that reversely magnetised rocks generally were magnetised by the Earth's field in a reversed polarity state. However, a few examples of self-reversal of the NRM in volcanic rocks have been reported: the Haruna dacite in Japan¹⁷, certain oceanic basalts^{18,19} and a basalt from Germany²⁰. Partial self-reversal leading only to a reduction of NRM intensity at room temperature, but not to a completely antiparallel alignment of the NRM direction with respect to the ambient geomagnetic field, was observed in an historical lava flow of Mount Etna²¹. Furthermore it has been shown in continental basalts^{22–24} that thermoremanent magnetisation (TRM) produced during moderate heat treatment in the laboratory can acquire self-reversal or at least partial self-reversal characteristics at room temperature.

Self-reversal processes can be classified into three categories according to different physical mechanisms: (1) A one-constituent model in which the direction of spontaneous magnetisation changes sign at a certain temperature (Néel's²⁵ P- or N-type ferrimagnets). (2) A two-constituent model in which negative exchange interaction across the boundary of two magnetic phases plays the essential role. (3) A two-constituent model in which magnetostatic interaction between two phases leads to complete or partial self-reversal of remanence.

I have sampled the reversed basalts of Laschamp (site F³) and Olby (site B³), and one normally magnetised flow to the west of Puy de Barme. The NRM directions obtained after alternating field cleaning are similar to those observed by Bonhommet²⁶, but also some normal samples have been found in the reversed

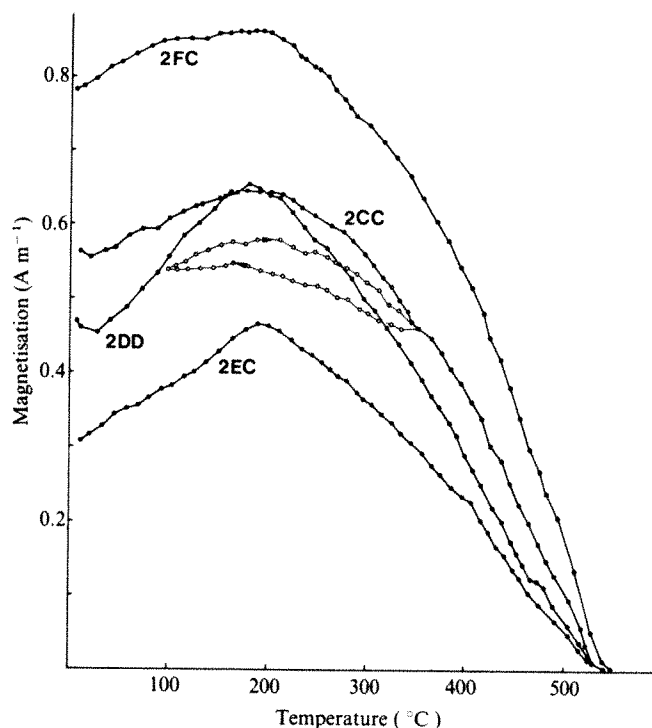


Fig. 1 Partial self-reversal of natural remanent magnetisation during heat treatment in four basalt samples collected from the reversed Olby flow. Heating rate, $10^\circ\text{C min}^{-1}$.

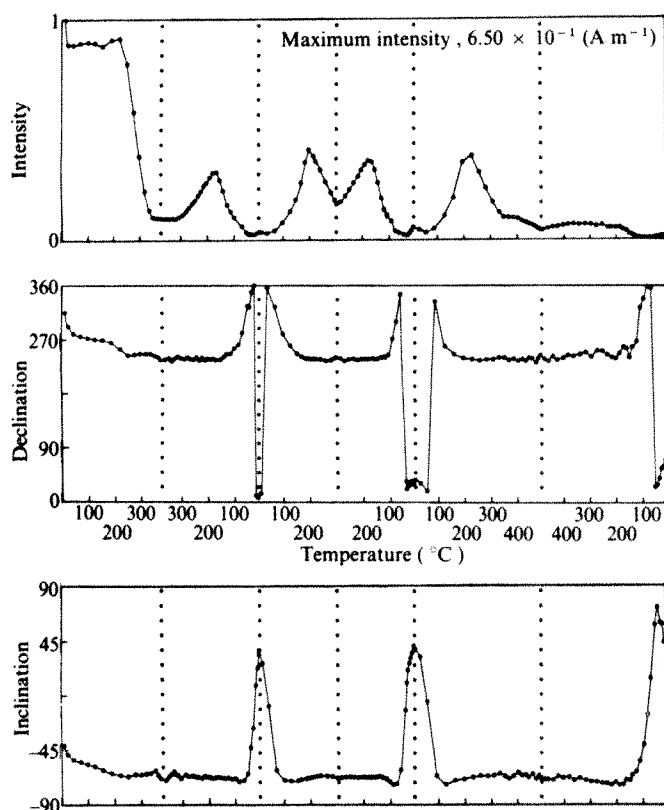


Fig. 2 Complete self-reversal of natural remanent magnetisation of an Olby basalt sample (QB 2 BC) during thermal treatment. Inclination, declination and intensity have been plotted separately against temperature. Three heating and cooling cycles follow each other. Note, the sharp peaks of the intensity curve around 200 °C are caused by the self-reversal process which leads to a normal remanence direction below 50 °C in all three cooling cycles. Above 200 °C the remanence direction is reversed and antiparallel to the room temperature direction.

Olby flow. Rockmagnetic studies by Whitney *et al.*²⁷ reported that both reversed lavas are highly oxidised without any indication of a self-reversal of NRM. My observations are that the rock-magnetic properties and other physical parameters (for example, oxidation state, porosity) of the lavas investigated vary to a large extent from flow to flow as well as within the flows.

The magnetic properties of the reversely magnetised Olby flow are particularly interesting and show that the NRM of at least this flow has self-reversal characteristics. Several samples of the flow have been subjected to continuous thermal demagnetisation²⁸. Figure 1 shows the NRM intensity behaviour during thermal treatment of four Olby samples which, before this experiment, were a.f.-cleaned in fields of up to 10 mT to remove secondary soft components. Up to 200 °C all samples show a peculiar increase in intensity amounting to up to 50% of the initial value. No directional NRM changes are observed during heating up to 550 °C. Sample 2CC shows that the demagnetisation curve is repeatable between 100 and 350 °C when the temperature is lowered and raised again. Therefore the initial intensity increase can be explained only by a partial self-reversal process acting in these samples. Below 200 °C an apparently normal NRM component is coupled to the pre-dominant reversed magnetisation the intensity of which is reduced in this way at room temperature.

Using the same demagnetisation technique as in Fig. 1, a complete self-reversal of NRM has been found in another Olby flow sample (Fig. 2). During the first heating a soft, viscous magnetisation component of normal polarity is removed up to 200 °C. This component obscures the behaviour of the stable NRM components in this temperature range. Further heating to 400 °C reduces the intensity, but leaves a stable reversed NRM direction which has a southwesterly declination and steeply

negative inclination as found often in the Olby flows²⁶. When cooling from 400 °C to room temperature in zero magnetic field ($H < 20$ nT) at first an intensity increase is observed till 170 °C which is followed by a sharp decrease in NRM intensity. At 150 °C the declination component begins to change direction. At about 40 °C the magnetisation changes sign and a normal NRM is built up roughly antiparallel to the former NRM direction. The same features are recognised in the subsequent second and third heating and cooling cycle (Fig. 2), where, during heat treatment, the NRM changes sign at temperatures between 50 and 70 °C. Thus, although their magnetisation behaviour is different, all three cycles lead to a complete self-reversal in this Olby flow sample.

At the present stage of investigation a unique answer to the self-reversal process itself cannot be given: one-phase or two-phase models may apply. On the basis of ore microscopic observation and thermal demagnetisation characteristics of NRM a two-constituent model is currently preferred. In contrast to earlier findings²⁷ I have optically identified only low temperature oxidised titanomagnetite in these Olby flow samples. Therefore the thermomagnetic curves may be controlled by two magnetic phases with different Curie temperatures (T_c): a titanomagnetite with $T_c \approx 180$ °C and a titanomaghemite with $T_c \approx 350$ °C (see Fig. 2). The sharp drop in NRM intensity during heating and cooling around 200 °C is caused by the reversal process, but seems to be incompatible with P-type magnetisation curves because of its peaked appearance. The disappearance of the sharp magnetisation peak at 200 °C during cooling in the third cycle may indicate that the Ti-maghemite has been destroyed by heating to near 500 °C. Thus a negative magnetic interaction between two phases may cause complete or partial self-reversal of NRM in experimental conditions. It still has to be shown that such an interaction took place during the original NRM formation. However, if the titanomagnetite formed at temperatures below 180 °C by low temperature oxidation, such a process would lead to a reversed NRM direction in the Olby flow in the presence of a normal geomagnetic field direction.

The reversely magnetised Laschamp flow carries highly oxidised titanomagnetite and complete self-reversal of NRM during laboratory experiments has not yet been proved. However, this lack does not dismiss the possibility of self-reversal for this flow, in particular as recent investigations²⁹ have demonstrated that self-reversal may occur in strongly oxidised titanomagnetite, but may not be reproducible by laboratory experiment.

The present experiments recall strongly the need for thorough rock-magnetic investigations³⁰ when ascribing reversed magnetisation directions in igneous rocks to reversals of the geomagnetic field.

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Palaeomagnetic constraints on Greater India's underthrusting of the Tibetan Plateau

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A minimum estimate of the magnitude of Greater India's underthrusting along the Main Central Thrust beneath its former leading edge and beneath the Tibetan Plateau to the north is reported here. A comparison of newly obtained late Palaeozoic and Mesozoic palaeomagnetic results from the Tibetan Sedimentary Series (Thakkhola Graben, north central Nepal) with results from the Indian subcontinent indicates an excess anticlockwise rotational movement of Greater India with respect to its former leading edge of 10–15°. Assuming that the pivot point for this differential rotation is located to the west of the Western Himalayan Syntaxis, this magnitude corresponds to a minimum underthrusting of continental lithosphere of 200–350 km at the longitude of central Nepal.

Large scale underthrusting of Greater India beneath the Tibetan Plateau has been postulated repeatedly on geological^{1–3} and geophysical grounds^{3,4} to explain the thickness of the crust beneath the Tibetan Plateau, about twice as thick as the average continental crust. This postulate conflicts with the established notion that buoyancy constraints^{5,6} preclude large scale subduction of continental lithosphere. Recent studies⁷ suggest, however, that such subduction over several hundred kilometres may be possible within the range of plausible values for model parameters.

The magnitude of Greater India's underthrusting beneath Asia can be constrained palaeomagnetically. Favourable conditions are: (1) northwards-directed underthrusting proceeded at first beneath Greater India's former leading edge which is preserved at the surface and can be studied; and (2) underthrusting resulted largely from an anticlockwise rotational

movement whose magnitude can be determined relatively easily. Besides, understanding of the evolution of the India–Asia collision has advanced considerably in recent times. Mutually supporting geological data^{3,8,9}, seafloor spreading analyses^{3,10,11}, and palaeomagnetic data^{12–14} indicate conclusively that welding of India to Asia along the Indus–Tsangpo suture zone (Fig. 1) occurred from about the late Palaeocene until maybe the late Eocene. Continental subduction during this phase was probably only of a minor magnitude^{3,8}. After the intimate contact had been established, the Indus–Tsangpo suture zone became immobilised and the India–Asia convergence slowed down considerably. A new zone of weakness developed about 100–200 km further to the south within Greater India and this zone evolved into the Main Central Thrust. Renewed convergence of intracontinental character occurred along the Main Central Thrust since possibly the Oligocene^{9,11}. Greater India thus underthrust its former leading edge and beyond, beneath the Tibetan Plateau. Powell and Conaghan^{3,4} postulated initially that Greater India's underthrusting may have proceeded beneath the full width of the Tibetan Plateau (over ~1,500 km at the longitude of far eastern Nepal). Powell¹¹, however, has recently reduced this estimate considerably.

Seafloor spreading analyses^{10,11,15–17} show that post-collisional convergence of India with Asia was characterised by an appreciable anticlockwise rotational movement of the Indian plate with respect to Asia. This can be interpreted as the main cause for the development of the pronounced asymmetrical pattern plan of the Himalaya^{11,18,19}. Our estimate for the magnitude of underthrusting along the Main Central Thrust is based on a comparison of newly obtained palaeomagnetic results from the former leading edge of Greater India with palaeomagnetic results from the Indian subcontinent (summarised in ref. 14). This method is particularly suited for the determination of the rotational component of underthrusting, but is much less precise in the determination of a possible northwards translational component of underthrusting.

Palaeomagnetic results representative of Greater India's former leading edge were obtained from late Palaeozoic and Mesozoic carbonates and sandstones from the Tibetan Sedimentary Series of the Thakkhola Graben, North Central Nepal. This region was chosen because of its relatively easy access, its detailed geological mapping^{20–22}, and the much lower degree of metamorphism than is prevalent in adjacent regions of the Tibetan Himalaya. Moreover, the Main Central Thrust is parti-

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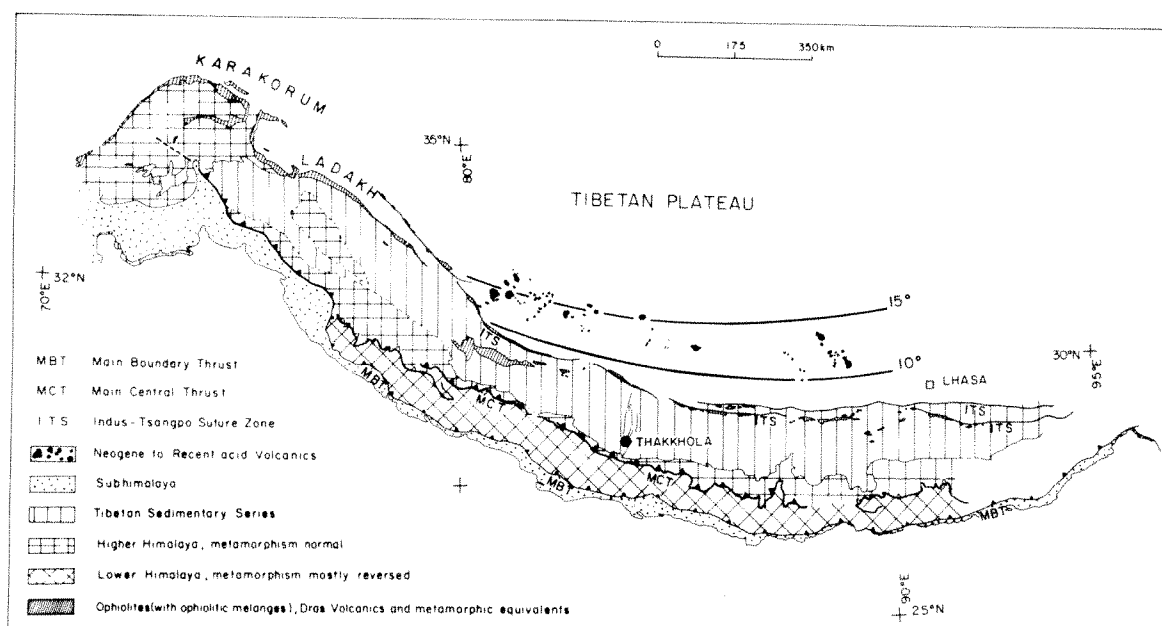


Fig. 1 Main structural features of the Himalaya after Gansser⁸. The dashed line indicates the westwards extension in the subsurface of the Main Boundary Thrust/Main Central Thrust complex according to seismic observations^{30,31}.

Table 1 Summary of Palaeomagnetic directions

Rock unit*	Age	N†	Dec(°)	Corrected for bedding Inc(°)	k	$\alpha_{95}(^{\circ})$	Fig. 2a Ref.
Secondary 'collision' component (50–60 Myr)							
Thinigaon Lst.		17	6.5	–9.5	39	6	1
Kagbeni Sst.		22	2	–12	16	8	2
Dzong Sst.		67	348	–6.5	23.5	3.5	3
Kagbeni Sst.		18	347.5	–5	16.5	8.5	4
Jomosom Lst.		77	346	–4	35	2.5	5
Jomosom Qzt.		36	346	–16	32	4.5	6
Primary component							
Dzong Sst.	Lower Aptian	78 (95)	326	–61.5	12	5	7
Kagbeni Sst.	Wealden	34 (40)	333.5	–56.5	15.5	6.5	8
Kagbeni Sst.	Wealden	24 (34)	322.5	–55.5	14	8	9
Lumachelle Fm.	Dogger	27 (43)	308	–61	12	8.5	10
Jomosom Lst.	Lias	22 (55)	308	–59.5	10.5	10	11
Jomosom Lst.	Lias	45 (82)	328.5	–42	14	6	12
Jomosom Qzt.	Rhaetian	32 (55)	323	–48	11	8	13
Thinigaon Lst.	Norian	22 (76)	324.5	–43	10	10	14
Thinigaon Lst.	Ladinian–Carnian	35 (57)	329	–47	21	5.5	15
Thini Chu Fm.	M?Permian	41 (40)	291.5	–63.5	10	7.5	16

Mean directions and statistical parameters were obtained according to ref. 38.

*Formation names and ages are according to refs 22, 28 and according to ref. 37 for the sampled part of the Thini Chu Fm.

†The number of specimens wherein the component concerned could be identified. The number of samples collected are in parentheses.

cularly well developed, and has been studied thoroughly²² in the region south of the sampled location.

A summary of significant palaeomagnetic data is given in Table 1; a more detailed account of results will be presented elsewhere (our work, in preparation). Altogether more than 750 core samples were drilled from cliff sections bordering the Kali Gandaki river in the tract between Shang (28°45'N 83°44'E) in the south and Kagbeni (28°49'N 83°46.5'E) in the north, and also in cliffs immediately north of the Muktinath sanctuary (28°48'N 83°50'E). We restricted our sampling mainly to the Mesozoic and to a lesser extent the late Palaeozoic carbonates and sandstones. These are very well exposed in a WNW–ESE trending belt situated ~10–20 km north of the main range, formed by the impressive Dhaulagiri and Nilgiri–Annapurna massifs. Middle and early Palaeozoic carbonates exposed immediately south of the sampled region, that is, in the north-face of Nilgiri, show a southwards increasing metamorphism

associated with formation of the Main Central Thrust and have not been sampled for that reason.

In general two specimens per sample were thermally demagnetised, using furnaces as described by McElhinny *et al.*²³, in about 10 steps over a temperature interval determined on the basis of more elaborate pilot demagnetisation studies. The directional content of the specimens was analysed from Zijderveld plots²⁴. Directions of characteristic components were determined using a principal component analysis program adapted from Kirschvink²⁵. Simultaneous breakdown of a multicomponent system of directions was fairly commonly observed in the carbonates. Whenever such specimens failed to respond satisfactorily to the Zijderveld-type analysis we analysed them subsequently for cryptic components with non-unique blocking temperature spectra²⁶, following Kirschvink's²⁵ method. Unit weight was given to specimen directions throughout the statistical analysis.

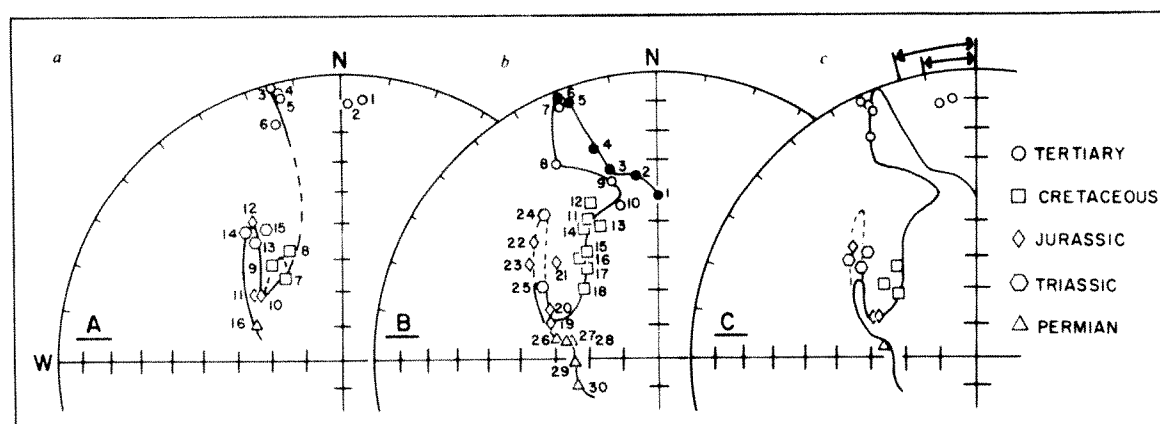


Fig. 2 Comparison of palaeomagnetic directions observed for the Thakkhola region (a) with directions expected for this region (b) according to palaeomagnetic data from the Indian subcontinent^{13,14} assuming that no relative movement between the two regions has occurred. The observed and the expected directions agree very well after correction for a 10° 'collision' component, 1–6) to a 15° (primary component, 7–16) differential rotation between the two regions (c). a, Secondary 'collision' component (1–6; 50–60 Myr) and primary components (7–16; Lower Aptian to Middle? Permian) as detailed in Table 1. b, 1, present field direction; 2, 5–22.5 Myr (DSDP); 3, 25–35 Myr (DSDP); 4, 35–45 Myr (DSDP); 5, 45–55 Myr (DSDP); 6, Sanjawi Lst. (Palaeocene–Eocene); 7 Upper Brewery Lst. (base Upper Palaeocene); 8, 55–65 Myr. (DSDP); 9, Upper Normal Deccan Traps (60–65 Myr.); 10, Lower Reversed Deccan Traps (60–65 Myr.); 11, 12, Tirupati beds (Lower Cretaceous); 13, 65–75 Myr. (DSDP); 14, Satyavedu beds (Lower Cretaceous); 15, Sylhet Traps (Lower Rajmahal Traps); 16, 17, Goru Formation–Parh Lst. (Aptian–Albian to Santonian–Campanian); 18, Rajmahal Traps (100–105 Myr.); 19, Loralai Lst. (Middle–Upper Jurassic); 20, Chiltan Lst. (Upper Jurassic); 21, Mean Middle Jurassic pole for Australia transferred to the Indian plate; 22, as above, mean Lower Jurassic pole; 23, as above for the mean Middle Jurassic pole of Antarctica; 24, Parsora beds (Upper Triassic); 25, Pachmarhi beds (Upper Triassic); 26, Kamthi beds, Wardha Valley (Permo–Triassic, 14); 28, Panchet beds (as above); 29, Kamthi beds, Wardha Valley (as above, 36); 30, Talchir beds (Permo–Carboniferous).

The specimens generally showed a fairly complex magnetic pattern. A recent field component of normal and to a lesser extent reversed polarity predominated, together with another secondary component and a primary component which were both mainly of normal polarity. Comparison with the Indian Tertiary apparent polar wander path (APWP)²⁷ shows that this secondary component stabilised between 50 and 60 Myr ago. This is also the time interval for which Krummenacher²² observed a notable resetting of K–Ar ages in central and eastern Nepal. Both the palaeomagnetic and the K–Ar data thus clearly reflect the initial collision of Greater India with southern Asia. Apart from the three magnetic components described above we also observed a west- to westnorthwestwards-directed secondary component of low downwards inclination and mainly of normal polarity. Its origin may be related to a similarly aligned pattern of *b*-axes^{22,28,29} which is well developed in the Tibetan Sedimentary Series. Secondary components of both normal and reversed polarity which originated during formation of the Main Central Thrust were dominantly present only in Carboniferous–Devonian rocks taken from the most southern sampling locality.

In the absence of contrary evidence we interpret the mean directions for the primary and secondary 'collision' components observed in the Tibetan Sedimentary Series of the Thakkhola Graben to be representative for Greater India's former leading edge, notwithstanding possible local distortions in its western extremity¹⁴. Figure 2 compares these mean directions (*a*) with the directional pattern expected for the Thakkhola region according to the Indian APWP (*b*). The observed and expected directional patterns agree very well after correction for an excess anticlockwise rotational underthrusting over 10° ('collision' component) to 15° (primary component) of Greater India with respect to its former leading edge (Fig. 2c). No clear indication for a northwards-directed translational underthrusting of Greater India beneath the Tibetan Plateau along the Main Central Thrust, or maybe along the Main Boundary Thrust³, was detectable in our palaeomagnetic results because of the inherent poor angular precision of such a determination. The asymmetrical pattern in the plan of the Himalaya^{17,18,19} indicates that the pivot point of the rotational underthrusting has to be looked for in the northwestern part of the Indian subcontinent. As a first approximation we assume its location at the western extreme of the Indus-Kohistan Seismic Zone^{30,31}. This zone represents the subsurface extension to the west of the Western Himalayan Syntaxis of the Main Boundary Thrust/Main Central Thrust complex, which thrusts, for all practical purposes, are indistinguishable from each other in the region immediately to the east of the Syntaxis. If we assume for the central and eastern part of the Himalaya that this differential rotation was restricted to rotational underthrusting along the Main Central Thrust alone, then its observed 10–15° magnitude corresponds at the longitude of central Nepal to a minimal underthrusting of 200–350 km of Greater India beneath its former leading edge and further north beneath the Tibetan Plateau.

As summarised by Molnar *et al.*¹⁹, geological, gravity and seismic data suggest an ~15° northwards dip of the Main Central Thrust. Assuming this dip to be uniform, the surface projection of the frontal part of underthrust Greater India can be located as shown in Fig. 1 according to rotational underthrusting values of 10° and 15°, respectively. Depths to the top of the frontal edge of this underthrust slab of continental lithosphere range from ~60 km in the west to ~120 km in the east taking the higher value of 15° of rotational underthrusting. It has been demonstrated³² that the calc-alkaline volcanism of island arcs is caused by melting of oceanic lithosphere at similar depths of 80–120 km. As Fig. 1 shows, there is a striking coincidence between the surface projection of the subducted slab of what in the present case happens to be continental lithosphere and a zone of Neogene to Recent calc-alkaline volcanism in southern Tibet. This zone is shown (Fig. 1) according to the 1:3,500,000 structural map of Gansser⁸, although other workers^{33–35} suggest a wider distribution of calc-alkaline volcanism. This coincidence supports our palaeomagnetic interpretation that continental

lithosphere can subduct in Himalayan-type conditions over distances of at least several hundreds of kilometres.

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A man-made hot spring on the ocean floor

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An instrument package emplaced in a deep-sea drill hole for the Glomar Challenger measured a rise in temperature at the bottom of the hole from 78°C shortly after drilling to 150°C after 42 days. The increase, not predicted by temperature measurements made before and during the drilling, is probably the result of hot water rising from below and flowing out of the hole into the ocean. We describe here this first recorded man-made hot spring on the ocean floor.

A downhole seismometer package was placed from the Glomar Challenger in DSDP hole 482-C (22°47.34' N, 107°59.57' W), located at the mouth of the Gulf of California about 12 km from the East Pacific Rise south of the Tamayo Fracture Zone¹. The hole was drilled in 3 km of water through 143 m of sediments and 47 m of massive basalt. The instrument (Fig. 1), constructed at the Hawaii Institute of Geophysics,

consists of a downhole sensor package containing thermal sensors, seismometers, tiltmeters, and associated electronics. Signals from the sensors are multiplexed and digitised into a 16-channel format for transmission by wire to a data recording and power package located on the ocean floor. The recording package is connected by floating rope to an anchor-float assembly that can be commanded to surface for data tape recovery and refurbishing of the system. A complete description of the experiment will be published elsewhere.

During the drilling, temperature was measured in the sediments with the Uyeda temperature probe, whose sensor is inserted into the sediments below the drill bit and is thus not affected by the drilling process. The temperatures show a linear trend (Fig. 2) compatible with the heat flow measurement of 12 HFU made during the site survey², if the conductivity of the sediments is assumed to be $2 \text{ mcal cm}^{-2} \text{ s}^{-1} \text{ }^{\circ}\text{C}^{-1}$. Using the same assumptions, the equilibrium temperature at the bottom of the hole should be between 90° and 110°C , depending on the conductivity of the basalt. As this value was below the design maximum of the instrument package (130°C), the sensor package was put in position on 3 February 1979.

During emplacement of the downhole seismometer package, temperature measurements were made during two time periods separated by 11 h. The first period was 30 min long and started about 7.5 h after the last water was pumped into the hole. The temperature at the end of this period was $20 \pm 2.5^{\circ}\text{C}$. By the end of the second period, 13.5 h later, the water temperature at the bottom of the hole had risen to $78 \pm 2.5^{\circ}\text{C}$, a rate of more than 4°C h^{-1} . This increase was assumed to be caused by the reheating of the hole after cooling by the drilling water.

After the second measurement period, the instrument was left to record data for 42 days and then recovered by the RV Kana Keoki on 17 March 1979. When the recording package was recovered, the sensor package was again monitored in real time. Although most of the electronics, including the multiplexer and the a to d converter were still operating, few of the sensors seemed to be working and there seemed to be little value in keeping the system operating. Therefore, the sensor package was removed from the hole. The package showed obvious signs of excessive heat; both tiltmeters had exploded, and

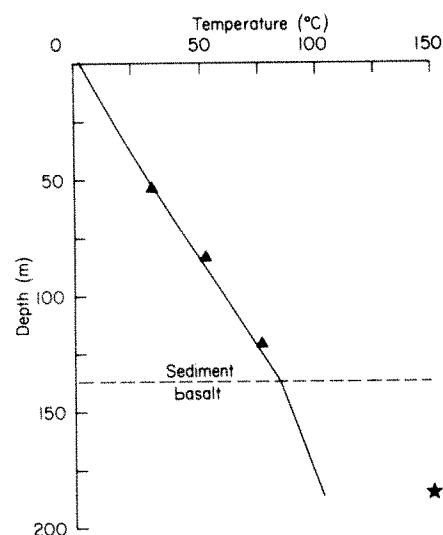


Fig. 2 Extrapolation of temperature measurements made while drilling (▲) yielded a temperature of about 100°C at the bottom of the hole. After 42 days, however, the temperature measured at the bottom (★) was about 150°C .

components and circuit boards that had been green before emplacement were now dark brown. Two components had mechanically shifted during emplacement, causing electrical short circuits through seawater to the recording package; however, the temperature sensors and circuitry were apparently undamaged. Although no data were obtained from the recording package, an additional 30 min of temperature information obtained during recovery showed that the temperature had risen dramatically during the time since emplacement. Because the temperature measurement obtained during recovery was greater than the maximum temperature for which the system had been calibrated (130°C), the sensor package was recalibrated at temperatures up to 155°C on its return to the Hawaii Institute of Geophysics. The new calibration values agree with those taken before the experiment up to 130°C ; the values above 130°C , while no longer linear (all components in the package were rated only to 125°C), were repeatable. Thus we believe that the $150 \pm 5^{\circ}\text{C}$ value obtained after 42 days in the hole is valid.

The discrepancy between predicted temperature at the bottom of the hole and the measured value can be explained if the hole acts as a conduit allowing hot water to rise from the more permeable basalt through the relatively impermeable sediments, forming a hot spring in the ocean bottom. The hole was probably heated over a period of time as hot water from below continued to enter the hole.

While there are other possible explanations for the temperature increase, we believe that the hot spring hypothesis is the most probable cause. Many authors have suggested that hydrothermal circulation is responsible for erratic heat flow values in the ocean³⁻⁹. Heat apparently is released in large amounts in regions where sediments are thin or where basement protrudes through the sediments⁵. In areas where sediments are thick, heat flow tends to be lower than expected because of the blanketing effect of the sediments. The theory of Parsons and Sclater¹⁰ predicts a value of 17 HFU for crust aged 0.5 Myr (hole 482-C) for conductive heat flow as compared with the measured value of 12 HFU, indicating that heat is being lost by means other than conduction even before the drilling. The drill hole is an efficient conduit whereby heat can flow through the low permeability sediments by convection. Recent discoveries of natural hot springs at ridge crests⁶ also document the importance of hydrothermal circulation in heat transfer near ridge crests and in the placement of hydrothermal ore deposits.

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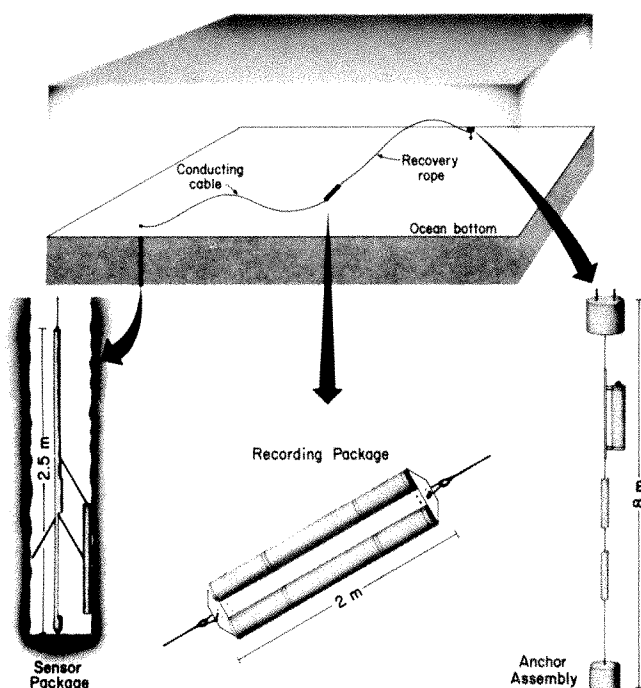


Fig. 1 The ocean sub-bottom seismometer. The sensor package was lowered to the bottom of the hole through the drill pipe by the Glomar Challenger. The pipe was then stripped from the wire and the recording package and anchor assembly attached and lowered to the ocean bottom.

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An Acheulean industry with *Elephas recki* fauna from Namib IV, South West Africa (Namibia)

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Namib IV is located in the linear dune sea of the central Namib Desert (Fig. 1), where different generations of fossil calcrete have been exposed by sand shift in an interdune flat. Mid-Pleistocene faunal remains (including *Elphas recki*) were scattered over 62,500 m² of the valley floor, some bearing residual traces of red calcrete. The industry may be closely paralleled from certain East African sites (Olorgesailie¹, Kilombe², PDK and HEB 1/2 and 3, Olduvai Bed IV (ref. 3)), suggesting a date between 400 and 700 kyr BP. I show here that Namib IV provides the first dated evidence for the presence of Acheulean man in South West Africa, the earliest Pleistocene faunal remains from the Namib Desert and a *terminus post quem* for the ecological change from savannah to sand desert.

Randomly orientated artefacts were scattered on the present ground surface, the presence of residual calcrete patches on the ventral surface of some artefacts and bones suggesting that they

had recently been weathered from a formerly extensive calcrete deposit of which a few remnants are still visible. Preliminary reconnaissance of the total distribution area was completed in 1978, and a statistical analysis made of 394 artefacts found within one randomly-selected grid square (22,500 m²), together with the collection of all faunal material from the site. The implement assemblage included bifaces (handaxes and cleavers) comprising 30% of the total, the rest being spheroids (2%), chopper/cores (7%), small flake tools (58%), working debris, débitage and unretouched flakes. Raw materials were very varied, principally yellow, pink and grey quartzite with lesser quantities of dolomite, dolomitic marble and diamictite obtained from cobbles in the canyon of the Kuiseb river, some 8 km north of the site. Some relationship between raw material and artefact type was evidenced, for example, by the dolomite spheroids (bolas) and the large cleavers which were almost exclusively made of quartzite. Smaller flake tools and scrapers were made from quartz, obtained from veins in the local outcrops of mica-schist.

There was a marked tendency for the bifaces to be made on flakes, a frequent method of manufacture being the splitting of a large pebble and the trimming of a few flakes from one side only, leaving the other consisting almost entirely of cortex. This was particularly evident on the cleavers, where as few blows as possible had been used consistent with producing a serviceable edge of the required shape. The handaxes, which tended to be pick-like or chisel-ended variants of the 'classic' pointed handaxe, composed only 5% of the whole industry. The cleavers, representing a further 25% of the industry, were larger and heavier with a marked tendency to be made on side-struck flakes. All bifaces showed traces of heavy usage.

The manufacture of bifaces from large flakes is characteristic of the Acheulean and it has been suggested⁴ that the development of the technique may be crucial in differentiating Acheulean industries from Oldowan. Typologically although Namib IV may closely be paralleled by East African industries from

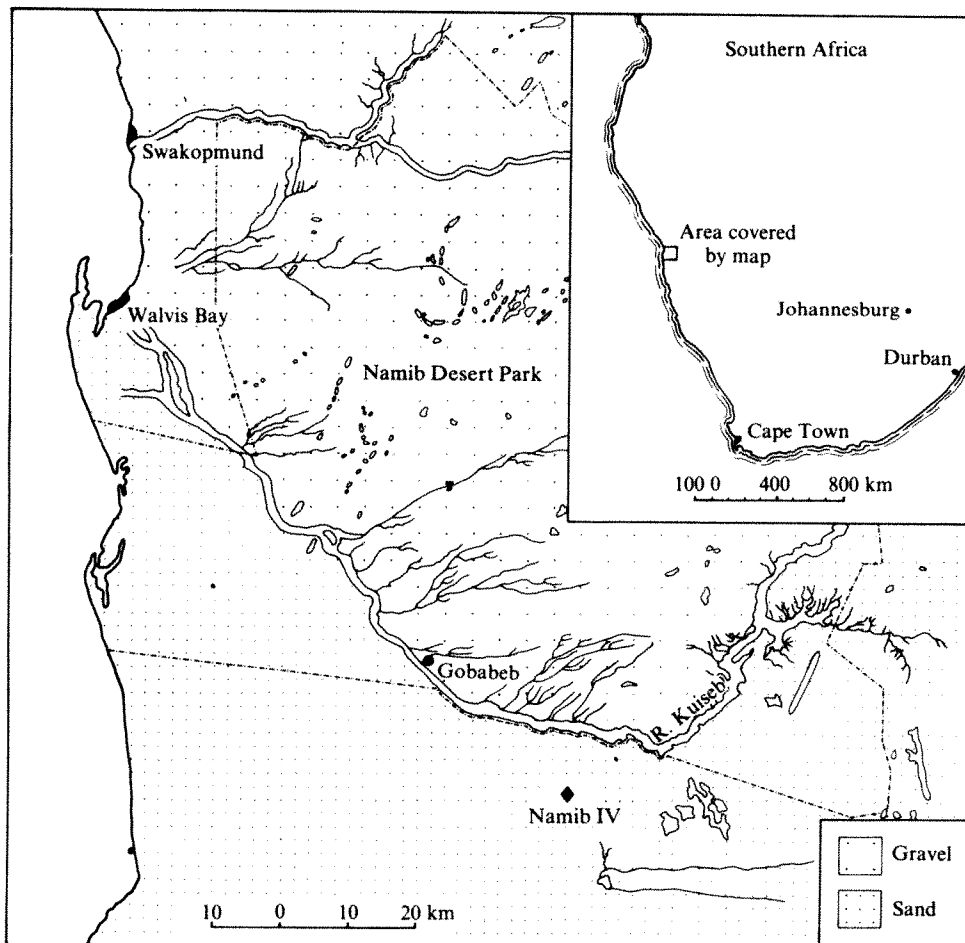


Fig. 1 Map of the central Namib Desert; inset shows its location in southern Africa.

Ologesailie, Kilombe and Olduvai IV, the thickness of the bifaces, low number of flake scars and lack of secondary working suggest that it might be slightly earlier in date, although firmly placed within the mid-Pleistocene Acheulean technocomplex. Certain features of the Namib IV assemblage, including the dominance of cleavers over handaxes, are in sharp contrast to the East African sites, although the complete absence of picks and the presence of sub-rectangular cores, spheroids and large number of flake tools reinforces the general similarity. Preliminary readings from Kilombe⁵ suggest that the site antedates the Brunhes-Matuyama transition, and is therefore >700,000 yr old. Isaac¹ has stated that it is likely that Ologesailie may date to before 400 kyr BP and it is suggested on typological grounds that Namib IV is of similar antiquity. The fauna included the mineralised remains of *Elphas recki*, together with one indeterminate alcelaphine antelope and one other medium-sized antelope. The *Elephas* remains fortunately included tooth fragments whose thin enamel, hypsodonty and tight enamel folding suggest that a late stage of *E. recki* is represented, probably comparable with Maglio's Stage 4 (ref. 6) as represented at Olduvai Bed IV, confirming the mid-Pleistocene date for the site.

The precise mode of formation of the calcrete deposit is as yet uncertain but it is undoubtedly connected with the former presence of surface water, probably a small ephemeral lake. The climatic shift which took place after the mid-Pleistocene resulted in an ecological change from savannah grassland with sufficient fodder for elephants to the present sand desert; a desiccation that resulted in a lowered water table and the formation of evaporites incorporating artefacts and bones lying on or near the former lake margins. The paucity of the bone refuse makes it impossible to say whether the artefact concentration represents a butchery site or a camp, but it is unlikely that hunters would have remained in the vicinity of their kills for more than a few days, due to competition from other, larger carnivores⁷. A butchery site visited on many occasions is the most likely hypothesis, an argument based on what Perkins and Daly⁸ called a 'schlepp effect', namely that bones which are bulky or of little nutritive value are unlikely to be removed from kill site to camp. Klein⁷ notes that the occurrence of elephant bones at Pleistocene base-camp sites in Africa is very rare, and that they are almost totally absent from the extensive faunas studied from South African cave sites. Acheulean butchery sites like Elandsfontein 'Cutting 10'⁷ are, conversely, often rich in elephant remains and sometimes, as in the case of Namib IV, these come from adult or sub-adult individuals.

Although the occurrence of Acheulean material in the Namib has been previously noted⁹ no associated fauna was preserved. It is hoped that an extension of the 1978 study of Namib IV will reveal typological and distribution information concerning the remains of the Acheulean in South West Africa, together with further details of the Pleistocene environment.

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In vitro flowering of embryoids derived from mature root callus of ginseng (*Panax ginseng*)

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Seedlings of various crops pass through a juvenile phase during which they cannot be induced to flower¹. The delay in flowering caused by a long juvenile phase may last for years and is a major problem in breeding such crops. In field conditions, ginseng (*Panax ginseng* C. A. Meyer) usually flowers in the third year after germination², and breeding programmes have been hindered by the long time needed for genetic analysis. We report here a repeatable precocious flowering of embryoids derived from mature ginseng root callus cultured in a chemically defined medium. These embryoids produce flowers and fertile pollen without establishing normal seedlings.

In vitro somatic embryogenesis of ginseng was first reported by Butenko and her colleagues³, who found that spontaneous somatic embryogenesis occurred on cultured callus derived from various tissues, including leaf, petiole, anthophore and stem. However, attempts to regenerate whole ginseng plants from these embryoids failed. We have since reported conditions for such regeneration⁴.

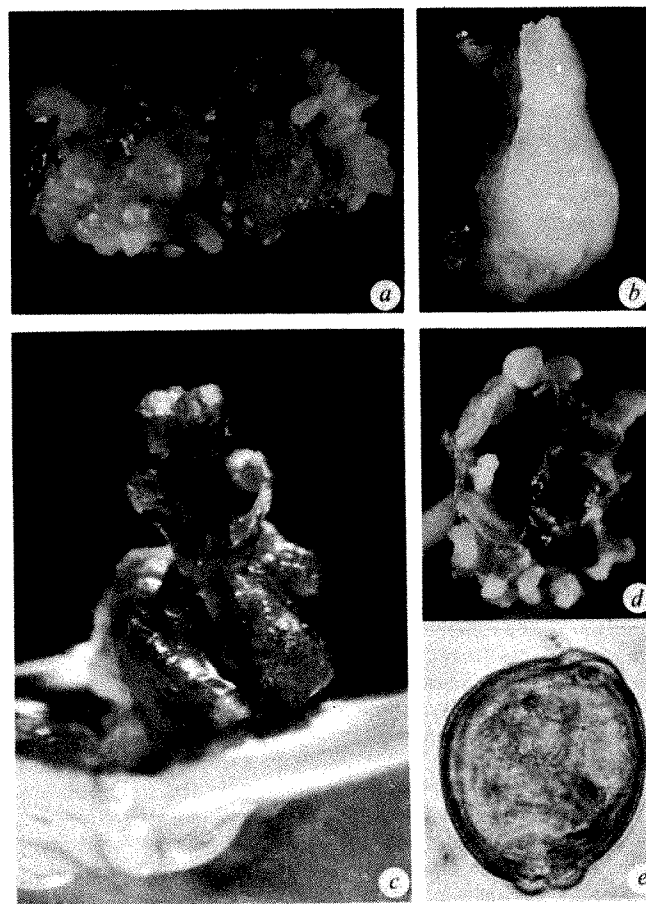


Fig. 1 a, Cluster of embryoids derived from mature root callus ($\times 5$); b, a dicotyledonary embryoid ($\times 40$); c, flowers developed from an isolated embryoid ($\times 4$); d, a minuscule flower with well-developed anthers ($\times 10$); e, fertile pollen of well-developed flowers ($\times 500$).

Callus was obtained by aseptically culturing pith tissue of a mature ginseng root on an agar medium consisting of 2% sucrose, vitamins and the major and minor salts of Murashige and Skoog⁵. This basal medium (BM) supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D, 5 mg l⁻¹) kinetin (2 mg l⁻¹). The cultures were kept in a growth chamber at 26 ± 1 °C in darkness. When subcultured, the callus grew most rapidly in BM plus 2,4-D (2 mg l⁻¹), kinetin (1 mg l⁻¹) N⁶-(Δ²-isopentenyl)-adenine (2iP, 1 mg l⁻¹). Embryoids were obtained by reducing the 2,4-D to 1 mg l⁻¹ and omitting kinetin and 2iP (Fig. 1a, b). They failed to develop and remained 2–3 mm long. Some of them, however, gave rise to a mass of friable callus, which produced more embryoids when subcultured on BM plus gibberellic acid (GA), adenine sulphate, tyrosine and charcoal; the optimal combination of these factors was 0.1 mg l⁻¹, 40 mg l⁻¹, 50 mg l⁻¹ and 0.5% respectively. Such embryoids, however, failed to develop further in subculture.

To obtain further development, embryoids 2–3 mm long were isolated from the callus and subcultured either on half-strength BM supplemented with benzyladenine (BA, 1 mg l⁻¹) and GA (0.5–1 mg l⁻¹) or B5 medium⁶ supplemented with BA (1 mg l⁻¹) and GA⁷ (1 mg l⁻¹). Well-structured flowers were repeatedly observed when the embryoids were cultured on the supplemented B5 medium at 26 ± 1 °C under a 16:8 light-dark regime, 1,500 lux (Fig. 1c). Two cotyledons of the cultured embryoid developed into a mass of green unorganised tissue, and the apical meristem located between two cotyledons transformed

into a peduncle terminated by a simple umbel with 3 to 15 flowers. These flowers were small, about 2–3 mm across. Each flower consisted of five-toothed green calyx, five yellowish green entire petals, 5 to 10 short stamens with oblong anthers, and two green pistils. These well-structured floral parts were minuscule but easily recognisable (Fig. 1d). About 90% of the pollen grains (Fig. 1e) were fertile as revealed by smearing and differential staining by Alexander's procedure⁷.

Thus embryoids have the potential to produce flowers directly in defined conditions. Compared with the intact plant systems used in studies of flowering, these embryoids have the advantage that many of them can be obtained in defined sterilised culture⁴, and within 1 month of subculture, as described here, flowers can be obtained. Thus the embryoid system could provide experimental material for studies of flowering without the need for the normal ginseng juvenile phase (3 yr).

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Luteinising hormone-releasing and anti-fertility properties of a glucagon-selective somatostatin analogue

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The hypothalamic tetradecapeptide, somatostatin (SRIF), inhibits the secretion of growth hormone (GH)¹ and numerous other hormones², including insulin and glucagon^{3,4}. Attempts to use SRIF as an adjunct in the treatment of diabetes mellitus^{5,6} met with limited success due to its short biological half-life^{7,8} and the undesirable diabetogenic activity of its insulin-lowering properties^{3,9,10}. Efforts at synthesis have yielded SRIF derivatives with prolonged GH-lowering activity which did not suppress glucagon or had equivalent insulin-inhibiting activity^{11–14} as well as several short-acting compounds with the appropriate glucagon specificity^{15,16}. A dodecapeptide analogue [des-Ala¹, Gly²] His^{4,5}-D-Trp³-SRIF (Wy-41, 747) has been identified that combines selective inhibition of GH and glucagon release with prolonged activity^{17,18}. However, in routine pharmacological tests chronic treatment of mature rats with Wy-41, 747 produced anti-reproductive effects resembling those described for luteinising hormone (LH)-releasing hormone (RH) and its agonists^{19–25}. We report here that Wy-41, 747, unlike SRIF and other of its analogues tested, releases LH, induces ovulation and inhibits pregnancy when administered before or after implantation; these properties are traditionally associated with the separate LH-releasing class of peptides.

Wy-41, 747 was administered to ten adult male and ten adult female (initiated on metoestrus of 4-d cycle) rats at a total dose of 900 µg per kg, given subcutaneously in three equally divided injections at 0900 h, 1500 h and at 0900 h on the following day.

A further group of ten males received SRIF in a total dose of 4.3 mg per kg, in view of its lesser potency and shorter half-life, delivered as six divided doses at 0900 h, 1000 h, 1500 h, 1600 h and 0900 h and 1000 h on the following day. Animals injected with vehicle (0.9% saline adjusted to pH 4.0 with HCl) served as controls. One hour after the final injection, serum was collected for radioimmunoassay of reproductive hormones^{21,26}. Wy-41, 747 caused a 2.9-fold increase in LH in males (control, 70 ± 15 ng ml⁻¹; Wy-41, 747, 203 ± 27 ng ml⁻¹) and a 21.9-fold increase in females (control 21 ± 4 ng ml⁻¹; Wy-41, 747, 466 ± 80 ng ml⁻¹). SRIF released no LH in males. Serum prolactin levels were reduced, although not significantly, by Wy-41, 747 in both sexes. The analogue depressed testosterone significantly in males ($P < 0.05$) but had no effect on oestradiol and progesterone in females. Thus Wy-41, 747, unlike its parent molecule SRIF^{2,27,28}, can release LH effectively and may also inhibit the release of prolactin as well as gonadal steroidogenesis.

Confirmation of the LH-releasing properties of Wy-41, 747 was provided by its ability to induce ovulation. At 1330 h on the day of proestrus, pentobarbital (50 mg per kg) was injected intraperitoneally to inhibit the spontaneous surge of LH necessary for ovulation. Test compounds were administered subcutaneously in saline, pH 4.0, between 1340 h and 1350 h and LH-releasing activity was determined by the presence of oviducal eggs the next morning (oestrus). LHRH was included as a standard by which to compare Wy-41, 747 and SRIF. As Fig. 1 shows, Wy-41, 747, unlike SRIF, induced ovulation in a dose-related fashion. Although very effective in this test, Wy-41, 747 was only 1/10 as potent as the endogenous LH-releasing peptide, LHRH. Preliminary evaluations of the ovulation-inducing activity for several other SRIF analogues indicate that the LH-releasing property is unique to Wy-41, 747.

A further group of pentobarbital-blocked proestrous rats was injected with the ED₁₀₀ dose of Wy-41, 747 necessary for ovulation (100 µg) and blood samples were taken by heart puncture 1 and 2 h later. Within 1 h the LH of the seven analogue-treated animals had increased to a level 6.6 times as great as that in three rats receiving saline; after 2 h the difference was 25.8 times. Wy-41, 747 also inhibited serum prolactin at both intervals after injection (1 h: saline control, 80 ± 37 ng ml⁻¹; Wy-41, 747, 19 ± 8 ng ml⁻¹; 2 h: saline control, 82 ± 32 ng ml⁻¹; Wy-41, 747, 11 ± 5 ng ml⁻¹).

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LHRH and its agonistic analogues, in spite of their fertility-enhancing classification (LH release, ovulation induction), consistently produce anti-fertility effects, including that of inhibiting pregnancy^{19,20}. Because this is presumed to be mediated by inappropriately increased endogenous LH levels^{21,22}, Wy-41,747, which possesses the LH-releasing property, was tested for its effect on pregnancy in rats. Beginning on day 1 of pregnancy (appearance of vaginal sperm) and continuing to day 7 (pre-implantational period), or beginning on day 7 and continuing to day 12 (post-implantational period), all compounds except SRIF were administered in a divided daily dose at 0900 h and at 1500 h. SRIF, because of its limited duration of activity, was administered in four divided daily doses at 0900 h, 1000 h, 1500 h and 1600 h. Autopsy and assessment of pregnancy status (the presence of at least one normal fetus was the criterion for pregnancy) occurred on day 14 (pre-implantation groups) or day 18 (post-implantation groups). Figure 2 shows that Wy-41,747 can terminate pregnancy when administered during either days 1–7 or days 7–12; in both tests it was approximately one-half as potent as LHRH. SRIF had little (pre-implantation) or no (post-implantation) inhibitory effect at high doses.

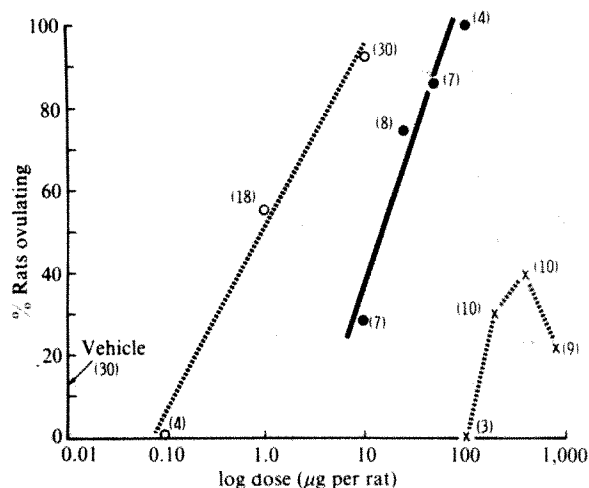


Fig. 1 Effect of LHRH (○), Wy-41,747 (●), and SRIF (×) on the induction of ovulation in pentobarbital-blocked proestrous rats. Numbers of rats are given in parentheses.

These results demonstrate that Wy-41,747, in contrast to SRIF and certain other SRIF analogues, has a reproductive pharmacological profile remarkably similar to that of LHRH. This SRIF derivative can release LH, induce ovulation and inhibit pregnancy before and after implantation, all integral properties of LHRH and LHRH agonists. Thus, the structural modifications of SRIF resulting in Wy-41,747 have resulted in a unique molecule with the high potency and the long-acting selectivity of the parent molecule (that is, ability to lower GH and glucagon) combined with characteristics of a separate class of peptides.

The anti-fertility effects of chronic administration of Wy-41,747, in addition to inhibition of pregnancy, include: in the male, decreased testicular and sex accessory weights, histological evidence of disorganised seminiferous tubules, inhibition of spermatogenesis; and, in the female, reduced folliculogenesis and uterine atrophy. These effects are strikingly similar to those reported for LHRH and its agonistic analogues^{21–25}. It has been proposed that the anti-reproductive action of LHRH/LHRH agonists occurs, in part, through an excessive release of LH, thereby reducing gonadal LH receptors (down-regulation) which is probably exacerbated by an associated decline in serum

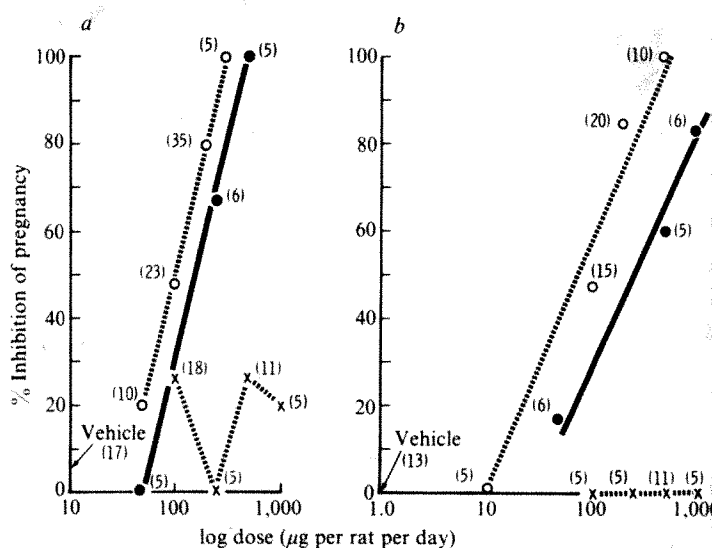


Fig. 2 Post-coital effect of LHRH (○), Wy-41,747 (●), and SRIF (×) in the rat. *a*, Inhibition of pregnancy as determined on day 14 after treatment during the days 1–7, pre-implantation; effect was characterised by lack of uterine implantation sites or resorbing sites. *b*, Inhibition of pregnancy as determined on day 18 after treatment during days 7–12, post-implantation; effect was characterised by resorbing sites and/or placental scars. Numbers of rats are given in parentheses.

prolactin levels^{21,22}. This, in turn, results in depressed gonadal steroidogenesis and a disruption of gonadal steroid-dependent tissues. The ability of Wy-41,747 to release LH and lower prolactin suggests that its anti-reproductive activity may occur through a similar pituitary-mediated downregulatory mechanism, although, as recently identified for LHRH and an LHRH agonist^{29,30}, direct gonadal effects may be involved as well.

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Proposed mechanism of cholinergic action in smooth muscle

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An increased turnover of phosphatidate and phosphatidyl inositol has been found in many tissues where hormones or neurotransmitters are postulated to raise Ca^{2+} influx¹, for example in smooth muscle²⁻⁴. However, the relationship between changes in phospholipid metabolism and changes in Ca^{2+} permeability was unknown. Following recent reports on the interactions of Ca^{2+} with phosphatidic acid in membranes and artificial systems, we investigated the hypothesis that phosphatidate accumulation mediates the action of cholinergic and other stimuli on Ca^{2+} influx. We report here that synthesis and accumulation of phosphatidate was accelerated in smooth muscle cells stimulated by carbamylcholine with a similar time course to that of contraction. This alteration in phosphatidate metabolism does not seem to result from an increase in intracellular Ca^{2+} or depolarisation of the cell membrane. Furthermore, submicromolar concentrations of phosphatidate rapidly produce contractions of isolated smooth muscle cells. These results support the contention that cholinergic-induced changes in membrane Ca^{2+} permeability in smooth muscle could be mediated by phosphatidate accumulation.

Several predictions of this hypothesis were evaluated. First, cholinergic stimulation should increase phosphatidate accumulation. Second, phosphatidate accumulation should occur independently of, and at least as rapidly as, the change in Ca^{2+} movement. Finally, exogenous phosphatidate should produce effects similar to those elicited by cholinergic stimulation. The use of enzymatically disaggregated smooth muscle cells from the stomach muscle of *Bufo marinus*⁵ afforded exceptional advantages in testing these predictions. The muscarinic response of those cells has been well studied⁶. Furthermore, changes in Ca^{2+} movements can be inferred from changes in the contractile state of the isolated cell, and their use obviates interpretive complications inherent to studies with intact tissues: heterogeneous cell populations, multiple diffusion barriers, and intercellular coupling⁷.

Changes in phosphatidate accumulation were assessed by following the incorporation of ^{32}P into the phospholipid. Within 10 s (a time when contraction is not yet maximal) after exposure to carbamylcholine, ^{32}P incorporation into phosphatidate was increased nearly threefold (Fig. 1). Similarly, brief periods of exposure to carbamylcholine increased phosphatidate content (assayed as glycerol-1-phosphate after saponification) from 340 to 570 pmol per aliquot cell suspension ($\sim 10^6$ cells). These data demonstrate that cholinergic stimulation increases phosphatidate accumulation, and this accumulation occurs rapidly enough to mediate changes in Ca^{2+} movement.

To assess whether the increased ^{32}P -phosphatidate labelling was independent of the increases in intracellular Ca^{2+} and/or the membrane depolarisation produced by cholinergic stimulation, the effects of K^+ on phosphatidate were studied. Exposure to high concentrations of K^+ produces contraction⁶ and would also be expected to depolarise the membrane. However, as K^+ failed to affect ^{32}P incorporation into phosphatidate, the effect of carbamylcholine on phosphatidate seems to be independent of membrane depolarisation and increased Ca^{2+} levels.

These studies are consistent with the hypothesis that increased phosphatidate accumulation may mediate the

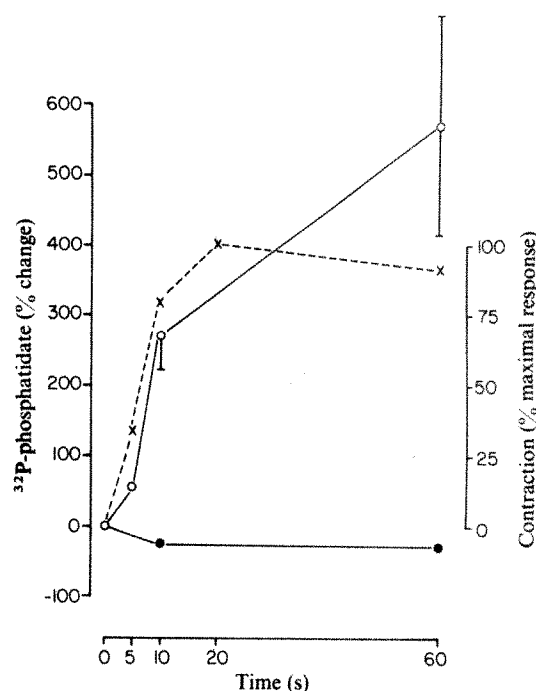


Fig. 1 Time course for the effect of carbamylcholine (carbachol) and KCl on ^{32}P incorporation into phosphatidate in isolated smooth muscle cells. For comparison the time course of carbachol-induced contraction is shown (data replotted from Fay and Singer⁶). Suspensions of isolated smooth muscle cells were incubated for 15 to 30 min in amphibian Krebs-Ringer bicarbonate buffer containing ^{32}P ($10\text{--}20\ \mu\text{Ci ml}^{-1}$) before exposure to carbachol (10^{-4}M) or KCl (40 mM) for the times indicated. The cells were disrupted by sonication (Branson cell disruptor) in the presence of 5% trichloroacetic acid and ^{32}P labelled phospholipids were isolated by two-dimensional thin layer chromatography on silica gel 60 (Merk) using solvent systems described by Abdel-Latif *et al.*¹⁸ ^{32}P -phosphatidate content was calculated from the specific activity of ^{32}P in the incubation medium. To correct for differences in cell density between experiments the increase in ^{32}P -phosphatidate produced by carbachol or KCl was expressed as a per cent change relative to ^{32}P -phosphatidate in unstimulated cells. Values for ^{32}P -phosphatidate of unstimulated cells ranged between 0.27 and 2.3 pg atoms per aliquot cell suspension. Note that carbachol increased ^{32}P -phosphatidate accumulation nearly threefold within 10 s (mean and standard error calculated from four experiments), a time where contraction is not yet maximal. Exposure of cells to KCl, which also causes contraction with a time course similar to that produced by carbachol, failed to increase ^{32}P -phosphatidate accumulation. Change in ^{32}P -phosphatidate: ○, carbachol; ● KCl; × contraction of carbachol.

increased Ca^{2+} levels produced by cholinergic stimulation. If this is so, then phosphatidate added exogenously should produce an increase in intracellular Ca^{2+} levels, hence contraction. Previous reports have shown that phosphatidate can elicit contractions in intact smooth muscle strips⁸, but such studies cannot rule out an indirect action (such as release of endogenous neurotransmitters). In isolated smooth muscle cells, phosphatidate at concentrations as low as 10^{-8}M produced contractions within 10 s. These contractions were equal in magnitude to those produced by carbamylcholine (Fig. 2).

As phosphatidate lies at an important branch point in glycerolipid metabolism, the ^{32}P labelling of phosphatidyl inositol noted by many investigators in other systems¹ may also arise as a consequence of increased ^{32}P -phosphatidate accumulation. The finding that carbamylcholine stimulates ^{32}P incorporation into phosphatidyl inositol in isolated smooth muscle cells subsequent to accumulation of ^{32}P -phosphatidate supports this contention⁴. It has previously been suggested that an increase in phosphatidyl inositol breakdown leading to an opening of 'Ca²⁺ gates' is the mechanism by which Ca^{2+} influx is accelerated by several

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agents^{1,9,10}. Although the present studies provide no information concerning the events leading to increased phosphatidate accumulation in response to carbamylcholine, it is likely that an accelerated hydrolysis of phospholipids, possibly including phosphatidyl inositol¹¹, is involved. However, the present studies define the time during which these events occur.

The way in which phosphatidate accumulation may initiate an increase in Ca^{2+} influx in the contractile response of smooth muscle to cholinergic agents can be inferred from other studies. As phosphatidate is an ionophore in artificial systems¹² it is reasonable to postulate a similar role in the cell membrane. The finding that phosphatidate monolayers are collapsed in the presence of Ca^{2+} , in effect removing the Ca^{2+} -phosphatidate complex from the interface, suggests the possibility that Ca^{2+} traverses the membrane complexed with phosphatidate, perhaps in the form of inverted micelles¹³. Furthermore, the accumulation of phosphatidate could increase membrane Ca^{2+} permeability without necessarily altering Na^+ or K^+ permeability since the affinity of phosphatidate for Ca^{2+} in aqueous media is 10^5 greater than that for monovalent cations¹⁴. Finally, if phosphatidate acts physiologically as an ionophore, then, to provide specificity in the control of membrane permeability, it would be expected to be ordered and segregated in regions of the membrane distinct from the bulk of zwitterionic phospholipids forming the bilayer. Numerous reports support the concept that phosphatidate forms electronegative domains, particles, or inverted micelles in membranes, especially in the presence of Ca^{2+} and basic proteins¹⁵⁻¹⁷. Thus, the association between changes in Ca^{2+} influx and changes in phosphatidate metabolism, noted in smooth muscle and many other tissues in

response to cholinergic and many other stimuli may be explained primarily by the generation of phosphatidate domains, functioning as Ca^{2+} 'channels'.

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Is phosphatidic acid a calcium ionophore under neurohumoral control?

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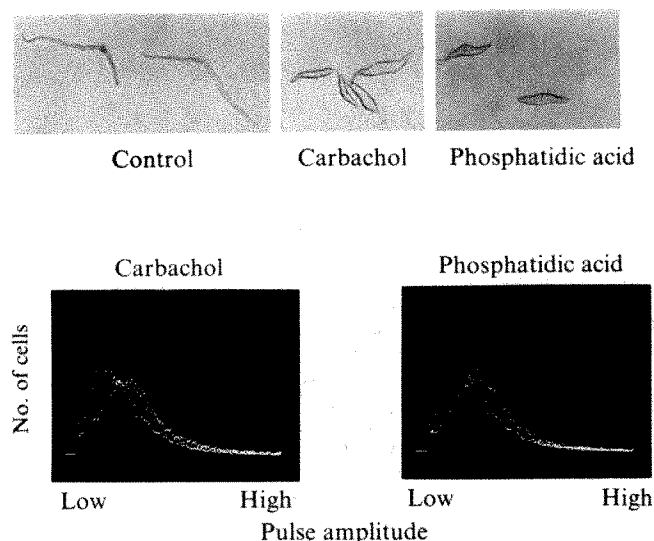


Fig. 2 Effects of exogenous phosphatidate on contractile state of isolated smooth muscle cells. Aliquots of single cell suspensions were exposed to 10^{-4} M carbamylcholine (carbachol) or 10^{-6} M phosphatidic acid (Sigma, grade I) for 15 s before fixation with acrolein⁵. Photomicrographs were taken at $\times 360$. The lower panel shows the pulse amplitude histograms obtained from 10^4 cells when aliquots of these same cell suspensions were analysed with a Coulter counter according to the method of Singer and Fay⁵. Note that both carbachol and phosphatidate produced contraction of the isolated cells such that cells length decreased by 60-70%. Phosphatidate-induced contraction was also noted when contractile state was assessed by Coulter counter analysis. Note that the pulse amplitude histogram generated by cells exposed to carbachol is shifted towards larger amplitude pulses when compared to the histogram generated by unstimulated cells. This increase in the number of large amplitude pulses is indicative of contraction of the isolated cells. A similar shift in the pulse amplitude histogram is noted for cells exposed to phosphatidate. In this experiment both carbachol and phosphatidate produced a 20% increase in the number of large amplitude pulses. In other experiments exposure of cells to phosphatidate at concentrations as low as 10^{-8} M or for as little as 5 s produced contractions of isolated smooth muscle cells.

There has recently been renewed interest in a previously observed phenomenon¹ whereby hormones and neurotransmitters alter the rate of incorporation of ^{32}P -phosphate into phospholipids, specifically, phosphatidylinositol (PI) and phosphatidic acid (PA)². This has arisen from a theory formulated by Michell and coworkers which postulates that this 'phospholipid effect' is in some way intimately involved in the mechanism by which certain neurotransmitters and hormones activate membrane Ca gates²⁻⁵. However, although Michell's observations are consistent with a phospholipid involvement probably somewhere between receptor occupation and Ca gating, they do not suggest what the specific role of the phospholipids in the Ca gating mechanism might be. Recently, Salmon and Honeyman suggested⁶ that the critical event may be the net formation of PA following the net breakdown of PI which occurs in pancreas⁷, platelets⁸ and smooth muscle^{6,9}. PA has been shown to behave as a Ca ionophore in a Pressman chamber¹⁰, and thus it is possible that an increase in PA concentration in cellular membranes might increase the Ca permeability of those membranes (see also ref. 5). In the rat parotid, receptors associated with Ca gating (muscarinic, α -adrenergic and substance P) show a phospholipid effect whereas β -adrenergic receptors (which act on adenylate cyclase) do not^{11,12}. The phospholipid effect is Ca independent, and is not produced by the divalent cationophore A23187 (refs 11, 12). Also, an easily quantifiable response of this tissue (K efflux measured as release of ^{86}Rb) is absolutely dependent on the concentration of external Ca (ref. 13) and, by inference, on the magnitude of Ca influx¹⁴. We report here evidence suggesting that PA, which is formed during the reaction sequence of the phospholipid effect, may directly mediate the inward movement of Ca that results from activation of surface membrane receptors.

Levels of PA were measured in dispersed parotid cells¹⁵ with two-dimensional TLC¹⁶. Unstimulated cells were found to contain 1.24 ± 0.15 nmol per mg protein PA ($n=6$). This was significantly ($P<0.005$) increased to 2.83 ± 0.37 nmol per mg

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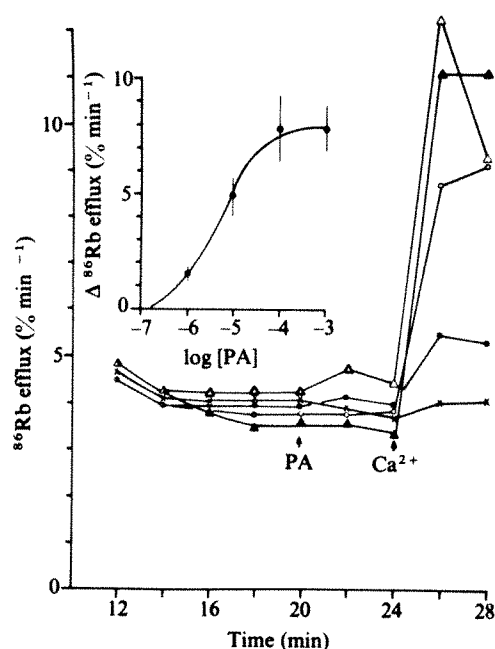


Fig. 1 Activity of phosphatidic acid (PA) in stimulating Ca-dependent ^{86}Rb release from rat parotid gland slices. The protocol of loading tissues with ^{86}Rb has been described elsewhere^{13,18}. The Ringer medium also contained 10^{-5} M atropine and 10^{-4} M phentolamine to prevent effects of endogenous neurotransmitter release on ^{86}Rb efflux. The Mg^{2+} concentration of this medium, and that for the experiments shown in Fig. 3, was 1.2 mM. This amount of Mg, about one-tenth of the K_a for Mg^{2+} , is not sufficient to interfere appreciably with Ca transport, but is necessary in the biological experiments to prevent membrane 'leakiness' that can occur on omission of extracellular Ca (ref. 22). Initially, the Ringer contained no added Ca and 10^{-4} M EGTA. At $t = 20$ min, phosphatidic acid was added to the media in the concentrations indicated, and at $t = 24$ min, CaCl_2 was added to a final concentration of 10 mM. Concentrations of PA (final) were: \times , none (control); \bullet , 10^{-6} M; \circ , 10^{-5} M; \blacktriangle , 10^{-4} M; \triangle , 10^{-3} M. The PA used was chromatographically pure ($\geq 99\%$), synthetic material (dipalmitoyl L- α -phosphatidate Na, Sigma P-4013). The values are means of three experiments each. The standard error averaged less than 10% of the means. Inset, basal ^{86}Rb efflux was subtracted and net increases in ^{86}Rb efflux at each concentration were calculated. Means of three experiments ± 1 s.e.m.

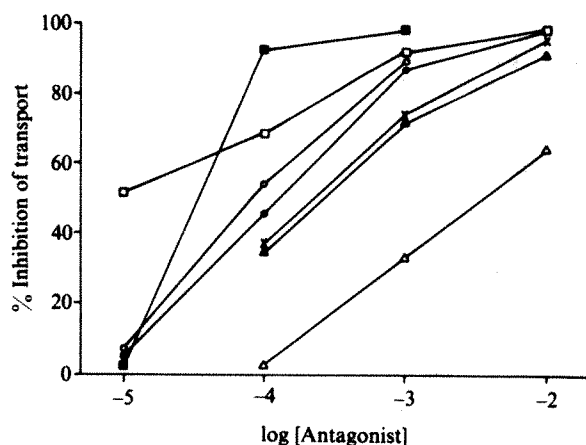


Fig. 2 Inhibition of PA-mediated ^{45}Ca transport into CHCl_3 by various agents. Equilibrium partitioning of ^{45}Ca (10^{-5} M) from 1.0 ml of Ringer medium (without Mg^{2+}) containing 10^{-4} M PA into 5.0 ml CHCl_3 was determined in the presence and absence of several agents at the concentrations indicated. The data are expressed as % inhibition; that is, 100% means no radioactivity in the CHCl_3 . The agents used were: \square , La^{3+} ; \blacksquare , Tm^{3+} ; \circ , neomycin; \bullet , Co^{2+} ; \blacktriangle , Ni^{2+} ; \times , Ca^{2+} ; \triangle , Mg^{2+} . The data summarise three separate experiments, each assayed in triplicate. The standard errors averaged 5% of the means.

protein PA ($n = 8$) on 30 min incubation with the muscarinic agonist, methacholine (10^{-4} M). Continued muscarinic receptor activation for this period of time produces a sustained activation of surface membrane Ca gates¹⁷.

Figure 1 shows the Ca-dependent stimulation of ^{86}Rb release from parotid gland slices by various concentrations of PA. Phosphatidylinositol and phosphatidylserine (another acidic phospholipid) had no effect at 10^{-4} M concentrations. Because the PA did not stimulate ^{86}Rb release until Ca was present, nonspecific effects on membrane permeability or an ability to transport ^{86}Rb directly can probably be ruled out. Also, as can be seen in Fig. 1, PA did not cause a transient response before the addition of Ca, as is typical for agents acting on endogenous receptors¹⁸. We conclude, therefore, that the action of exogenous PA is probably not mediated through endogenous receptors.

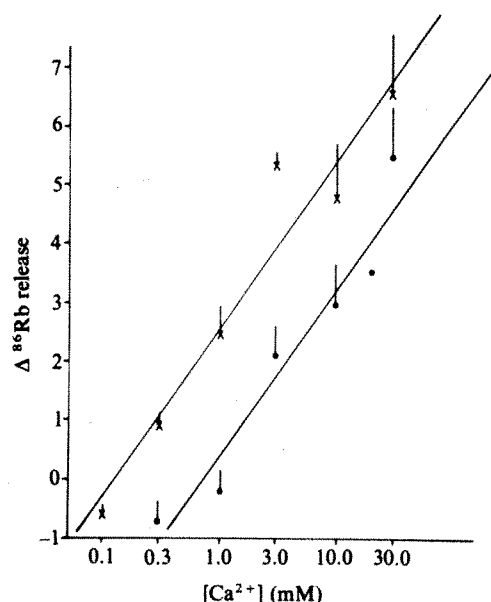


Fig. 3 Concentration-effect relationship for Ca-dependent ^{86}Rb release. The data were obtained from experiments similar in design to those shown in Fig. 1, except that the receptor-controlled Ca gating mechanism was activated with the muscarinic agonist, carbachol. To assure that Ca gating was rate limiting, a submaximal concentration of carbachol (10^{-6} M) was used¹⁸. Basal rates of ^{86}Rb release (before Ca was added) were subtracted. The linear portions of the curves were fitted by least-squares analysis, and the log-displacement of the curves at $y = 3\% \text{ min}^{-1}$ was used to calculate K_a values. \times , Control ($n = 4$); \bullet , plus 2 mM Co^{2+} ($n = 3$). The dispersions represent s.e.m.s. Other agents were tested with similar procedures and three or four replications each.

The assay of ^{45}Ca partitioning into CHCl_3 due to PA was carried out as follows: 1.0 ml of Ringer medium (with no Mg) containing 10^{-5} M ^{45}Ca and 10^{-4} M PA was shaken with 5.0 ml of CHCl_3 . After sufficient time for the system to come to equilibrium, the radioactivity in the organic phase was determined. In the absence of PA, virtually no radioactivity was detectable in the organic phase. With PA present, about 5% of the radioactivity originally added was extracted by the CHCl_3 . This effect was comparable to that seen with 20 μM A23187. Phosphatidylinositol and phosphatidylserine did not augment ^{45}Ca partitioning into CHCl_3 . Tyson *et al.*¹⁰ surveyed several phospholipids and found that only phosphatidic acid and cardiolipin produced this effect. The abilities of various agents to inhibit this process were determined by repeating the extraction in the presence of various concentrations of the appropriate reagents. The results are shown in Fig. 2. The relative potencies of the agents tested were in the order: $\text{La}^{3+} > \text{Tm}^{3+} > \text{neomycin} > \text{Co}^{2+} > \text{Ni}^{2+} \approx \text{Ca}^{2+} \gg \text{Mg}^{2+}$. Although the actual mechanism involved in the PA-induced partitioning of ^{45}Ca into

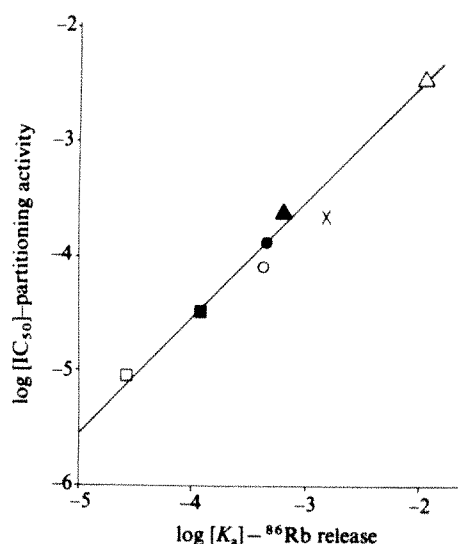


Fig. 4 Relationship between inhibition of ^{45}Ca -partitioning activity of PA and inhibition of receptor-activated Ca-dependent ^{86}Rb release by various agents. The ordinate values are concentrations of agent necessary to inhibit ^{45}Ca transport by PA into CHCl_3 by 50% as determined graphically from the data in Fig. 2. The abscissae are apparent dissociation constants (K_d) for antagonism of Ca gating in parotid slices determined as illustrated for Fig. 3. The agents were: \square , La^{3+} ; \blacksquare , Tm^{3+} ; \circ , neomycin; \bullet , Co^{2+} ; \blacktriangle , Ni^{2+} ; \triangle , Mg^{2+} ; \times , Ca^{2+} (K as agonist estimated). The line was fitted by least-squares analysis. Ca was omitted, however, because the K value, as an agonist, is somewhat uncertain. The slope (using logarithms) was 0.998 and the correlation coefficient was 0.993.

CHCl_3 is not known, it is reasonable to conclude that the potencies of these inhibitory cations in preventing this process reflects their ability to compete with ^{45}Ca for binding to the negatively charged head group of the PA. In addition, the Ca antagonist D-600, as well as procaine and tetracaine, were tested (not shown) and found to be somewhat less potent than Mg^{2+} .

The same agents were tested for their ability to inhibit endogenous, receptor-controlled Ca gating. The protocol here was to determine the Ca concentration-effect relationship for stimulating ^{86}Rb release (with carbachol as agonist) in the presence and absence of a fixed concentration of antagonist, and then to calculate antagonist dissociation constants from the shift in the Ca concentration-effect curve¹⁹. An example is shown in Fig. 3. The potencies for inhibition of Ca-mediated ^{86}Rb release (in the case of Ca, its apparent K value as agonist) ranked as $\text{La}^{3+} > \text{Tm}^{3+} > \text{neomycin} > \text{Co}^{2+} > \text{Ni}^{2+} > \text{Ca}^{2+} > \text{Mg}^{2+}$. Procaine, tetracaine and D-600 had no antagonistic activity below 10^{-3} M, and at higher concentrations produce inhibition by other mechanisms^{20,21}.

The correlation between inhibition of partitioning activity of PA and apparent affinity for the Ca influx mechanism (as assayed by ^{86}Rb release) is illustrated in Fig. 4. Taken with the observations that receptor activation increases the PA content of the parotid, and that exogenous PA can stimulate a Ca-dependent response in the parotid, these data suggest that PA may function in the physiological mechanism for Ca gating. This does not imply that PA must act in as simple a manner as for the ionophores in general; auxiliary structures and functional groups may also be involved. In red cells, for example, increased levels of PA do not apparently lead to augmentation of Ca influx⁵. As the red cell does not seem to have any Ca-gating mechanism under receptor control, this may indicate that other structures necessary for this phenomenon are also absent. However, in the parotid gland and other tissues, receptor mechanisms are present which regulate Ca gating as well as the membrane concentration of PA. On the basis of the data presented here, we suggest that the critical binding site for Ca

gating in these tissues could well be receptor-regulated phosphatidic acid.

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Motoneurone counts in *Xenopus* frogs reared with one bilaterally-innervated hindlimb

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The death of large numbers of young neurones is a striking feature in the developing nervous system. Many authors have postulated that the neurones are produced in excess of the numbers that their target tissues can support, forcing them to compete for survival¹⁻³. Observations on developing limb motoneurones (ventral horn cells) in both frogs and chicks seem to support this hypothesis. Normally, one-half to three-quarters of ventral horn cells die as limb movements begin^{4,5}, but some of these (up to 25% in chicks, 7% in frogs) can be rescued by the provision of supernumerary limb buds before motor axon outgrowth begins⁶⁻¹⁰. Conversely, amputation of the limb bud causes an increased death rate approaching 100% (refs 4, 11). The crucial test of the hypothesis depends on its prediction that the limb should limit the number of surviving motoneurones, and here I have attempted to make the test. For this, both sides of the spinal cord were forced to project to a single hind limb bud well before the onset of ventral horn cell death. It was found that the combined total of surviving motoneurones (right plus left sides) projecting to the single limb exceeded the number that projects to one limb in normal animals by up to ~100%. It is concluded that the limb does not limit the number of survivors, and therefore the hypothesis is refuted, at least for ventral horn cells. It now seems more likely that motoneurone numbers are determined either by the need to eliminate ventral horn cells not connected with appropriate limb regions, or by factors operating within the central nervous system.

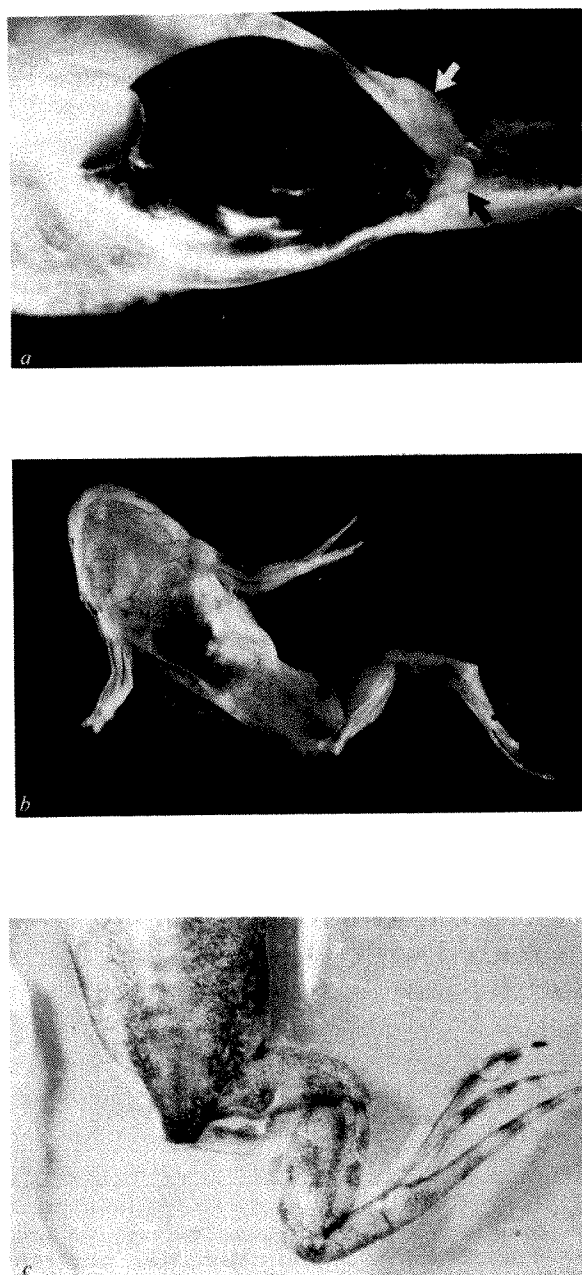


Fig. 1 *a*, Stage 49 tadpole (ventral view) after operation to move one limb bud (black arrow) into the midline. The other limb bud was first removed together with a small margin of surrounding epithelium. An incision was made through the tail fin caudal and dorsal to the anus and extended into the trunk along the dorsal surface of the peritoneal cavity, stopping just short of the renal duct. The flap of tail fin containing the anus (white arrow) was pulled across and fixed against the body wall with a fibrin clot to pull the remaining limb bud into the midline against the retroperitoneal wound. The flap returned to its normal position after 1 or 2 days, leaving the limb bud adhering to the new position. $\times 11$. *b*, Juvenile frog (M146) with a midline limb (ventral view). Photograph taken after fixation. This was the only experimental animal to have an abnormal limb, with two missing toes and a slightly reduced range of movement at the hip. $\times 2$. *c*, Juvenile frog (M213) without limb bud displacement (dorsal view). After amputating the other limb bud, a needle was used to tear the retroperitoneal region separating the two limb buds. Among the natural barriers to axon growth in this region are some large blood vessels which caused heavy bleeding. However, blood flow through nearby structures including the remaining limb seemed unaffected. $\times 7$. All operations were carried out in a solution containing 1 g sea salt, 4 g NaCl and 9 g l⁻¹ D-glucose plus 1 : 5,000 MS222. Tadpoles recovered in a solution containing 5 g l⁻¹ NaCl.

Operations were performed on 10–15-day-old *Xenopus laevis* tadpoles at stages 49 and 50 (ref. 12), when motor axon invasion of the limb buds is about to begin¹³. (Degeneration of ventral horn cells begins about a week later at stage 53, lasts 3–5 weeks until stage 60 and causes the loss of about 75% of the original population.) After removing one limb bud, the other was either left *in situ* or displaced into the midline, dorsal to the anus (Fig. 1*a*). Midline barriers which normally confine the spinal nerves to their own sides were disrupted with a needle. Only eight tadpoles survived the operations but they matured without delay. Limb buds regenerated in three tadpoles and were removed as they appeared.

In all except one frog with minor abnormalities (M146, Fig. 1*b*), the single hind limbs were normal in every respect including muscle bulk and amount and character of visually observable movements. None of the frogs had any trace of a limb, pelvic bones or muscles on the amputated side. To prove that in the metamorphosed frogs the limb was innervated by both sides of the cord, motoneurons projecting to the limb were labelled by retrograde axonal transport of horseradish peroxidase (HRP) either injected into the gastrocnemius muscle or applied to the proximal stump of the sciatic nerve after transection in the thigh¹⁴ (Fig. 2*a*). Further evidence of bilateral innervation was obtained at dissection 2 days later (7–14 days after completion of metamorphosis) by observing the spinal nerves from both sides entering the limb (Fig. 2*b*). The dorsal root ganglia were not preserved for cell counts but they seemed normal in size at dissection.

The numbers of motoneurons ipsilateral to the remaining limb were normal in all animals (Table 1). Contralateral motoneuron numbers varied from 11 to 94% of normal. Summing the two sides gives the total number of motoneurons projecting to the single limbs. In the cases with good bilateral innervation (over one-third contributed by the contralateral side) the totals ranged from 2,325 to 3,189, far exceeding the number of motoneurons projecting to one limb in control animals (range 927–1,704, Table 1). This result shows that the

Table 1

	Age at maturity (weeks)	Cell counts		Total	Left/right (%)
		Right (ipsi-lateral)	Left (contra-lateral)		
Experimental					
M188*	9	1,449	1,371	2,820	94
M146*†	10	1,668	1,521	3,189	91
M213†	7	1,353	1,155	2,508	85
M69	11	1,287	1,038	2,325	81
M208	7	1,617	1,119	2,736	69
M209	7	1,599	843	2,442	53
M68	11	1,140	321	1,461	28
M187*†	8	1,773	189	1,862	11
Controls					
Unoperated					
M160	8	1,458	1,497	2,955	103
M162	8	1,452	1,437	2,889	99
M163	8	1,632	1,572	3,204	96
Sham operated					
M167	8	1,440	1,404	2,844	98
M168	8	1,542	1,614	3,156	105
M169	8	1,587	1,521	3,108	96
Other operated					
M65	10	1,005	—	—	—
M66	10	927	—	—	—
M195	7	1,677	—	—	—
M198	7	1,704	—	—	—

Frogs M65–69, M146 and M160–213 were reared from three different batches of tadpoles of different parentage. Unoperated controls were raised in the same tank as M187 and M188. Sham-operated controls had midline barriers disrupted but no limb bud amputation. In two of these cases a few motoneurons were found to project contralaterally (2 and 16 contralateral HRP-labelled cells; one gastrocnemius injected). 'Other operated' controls had partial deletions of one limb bud at stage 50, but no disruption of midline barriers. Counts are from the unoperated sides.

* Limb bud displaced into the midline.

† Regenerating limb bud removed. All regenerates were less than 0.3 mm long on removal.

limb does not limit the number of motoneurons projecting to it at projection densities found in normal animals. It also seems unlikely that there is a limit at the higher densities achieved in this experiment. This is shown by the absence of any correlation between the proportion of the total (ipsilateral plus contralateral) motoneurone projection contributed by the contralateral side, and the ipsilateral motoneurone counts ($r = -0.073$).

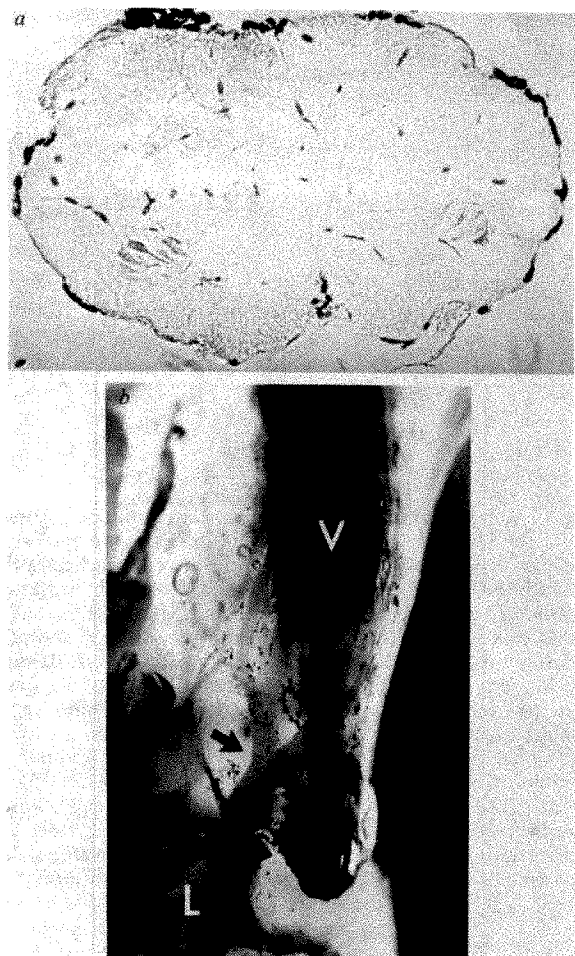


Fig. 2 Evidence for bilateral innervation. *a*, Transverse section of frog (M69) spinal cord showing HRP labelled motoneurons in both ventral horns. Bilateral labelling was also found in all other experimental animals except M146, which was not given HRP. In the case illustrated here, 30% HRP (Sigma VI) was applied to the transected sciatic nerve in the thigh. As in normal animals¹⁴, this method leaves about half the motoneurons unlabelled. Presumably, they project to the limb by other nerve trunks, or leave the sciatic nerve proximal to the transection. $\times 70$. *b*, Spinal nerves from both sides of the vertebral column (V) of M188 join to form one nerve trunk (arrow) before entering the base of the limb (L). $\times 14$. Spinal cords were dissected out and prepared for HRP histochemistry and cell counting by fixation in cold phosphate-buffered (pH 7.0) 2% glutaraldehyde for 4 h followed by overnight washing in running water. Cords were then immersed in 0.08% buffered diaminobenzidine tetrahydrochloride (Sigma) to which H_2O_2 had been added, after 1 h, to a concentration of 0.01%. Incubation at room temperature continued for 45 min before standard paraffin embedding for serial sections. Sections were mounted unstained in DPX and viewed by phase contrast microscopy. Counts were made immediately after mounting when cellular details, especially the nucleoli, are clearest. Motoneurons were identified using the criteria of Prestige⁴, which are based on position, alignment and size. Motoneurons with nucleoli were counted in every third section and no corrections were made for nucleolar splitting which probably occurs rarely⁴. Medial motor column neurones were not counted as they do not supply the limb. HRP labelling was assessed 2–3 weeks later after partial drying of the DPX to maximise visibility of the label.

Any competition should have shown as a negative correlation with ipsilateral counts falling as the contralateral projection increased. Conversely, the fact that ipsilateral counts were not increased above normal when the contralateral projection was small shows that the operation itself had not created a 'super-limb' with an increased motoneurone-supporting capacity.

Although it has been shown that the limb does not limit the number of survivors, a form of peripheral competition may still be postulated in which the survival rate is decided, not by the amount of space or trophic factors in the limb, but by a variable quality intrinsic to the ventral horn cells themselves. For example, using the analogy of territorial aggression, 'strong' ventral horn cells may acquire all the synaptic sites or trophic factors at the expense of the 'weak' cells. This idea was tested recently in *Xenopus* by allowing some ventral horn cells which normally die to have sole occupation of a part of the limb. Nevertheless, their deaths were not prevented and the part of the limb was left uninervated¹⁵, proof that at least some cell deaths are not due to peripheral competition. This evidence and the present results militate strongly against any form of peripheral competition determining motoneurone numbers.

Although the limb may not directly control numbers it remains indisputable in both *Xenopus*⁴ and chick⁵ that ventral horn cells must make contact with it to survive. Indeed, concurrent experiments in *Xenopus* (unpublished) have shown that the contact must be made with regions of the limb for which the motoneurons have been specified. This may provide one explanation for the present result. Specification may be so precise that only the minority of ventral horn cells succeed in finding their region, but all those that do, survive.

Control of motoneurone numbers may also be exercised outside the limb. An intriguing possibility is that the ventral horn cells may yet be competing (for example, for afferent connections, blood supply) but in the spinal cord, not in the limb. Some authors have also conjectured that not all ventral horn cells are presumptive adult motoneurons capable of long-term survival. Apart from some work showing that all ventral horn cells have axons at least as far as the ventral root¹⁶, this question has not been studied in *Xenopus*. However, extensive investigations in the chick embryo have not so far shown any differences between ventral horn cells destined to live or die¹⁷.

We are left with the problem of how supernumerary limbs prevent some ventral horn cells from dying^{6–10}. The usual interpretation is that the increased limb field allows more competing cells to survive^{2,7}. With peripheral competition now in doubt, this interpretation is less attractive. Others should instead be considered more seriously, including effects of the supernumerary limbs on ventral horn cell specification¹⁸, or on the central control of their survival. Tests of these possibilities should be feasible.

Other interesting questions arising from this study concern the electrophysiology of the motoneurons projecting to the single limb, how they distribute their innervation to the muscle fibres and the implications for theories of axonal guidance. In the three frogs given HRP into the gastrocnemius, normal projections were found from both ventral horns. Further evidence of normal somatotopy would argue that at least in *Xenopus*, axons are actively guided into the limb and not just channelled fortuitously by mechanical or geographical factors as recent work in the chick embryo seems to suggest^{19,20}. The single limb preparation should be an excellent model for studying various aspects of developing limb innervation.

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Opiate receptors influence vasopressin release from nerve terminals in rat neurohypophysis

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A previous report described the existence of substantial amounts of enkephalin immunoreactivity and the occurrence of nerve terminals containing an enkephalin-like material in the pars nervosa of rat pituitary¹. It was suggested that an enkephalin innervation of the pars nervosa originating from the magnocellular hypothalamic nuclei might regulate the secretion of neurohypophyseal hormones. The results of the present studies support this hypothesis, as we find that a stable enkephalin analogue (D-Ala², D-Leu⁵-enkephalin) inhibits the calcium-dependent release of vasopressin evoked by electrical stimulation of the rat pituitary stalk *in vitro*. A similar inhibition of the stimulus-evoked vasopressin release is caused by morphine and β -endorphin, and the inhibitory effects of the enkephalin analogue can be reversed by naloxone. These findings suggest the possible existence of inhibitory opiate receptors on the terminals of vasopressin fibres in the pars nervosa.

The release of vasopressin from isolated pars nervosa/pars intermedia tissue fragments was studied using a method similar to those previously described^{2,3}. The pars nervosa with attached pars intermedia tissue was dissected from adult male Sprague-Dawley rats (Charles River; 150-200 g) and incubated in 0.5 ml Krebs-bicarbonate medium at 37°C continuously gassed with 95% oxygen:5% carbon dioxide. The pituitary stalk was drawn into the tip of a silver wire suction electrode to allow electrical stimulation and during periods of stimulation the gland was raised to the surface of the fluid. The medium was changed at

Table 1 Calcium dependence and tetrodotoxin sensitivity of vasopressin release

	n	Arg ⁸ -vasopressin (pg equivalents per min)	
		Resting release	Stimulation-evoked release
Control	18	93 ± 24.7	1,703 ± 268.7
Ca ²⁺ -free	4	140 ± 46.7	60 ± 7.7
Tetrodotoxin 1 µg ml ⁻¹	4	127 ± 13.3	83 ± 18.0

Rat pituitary pars nervosa fragments were incubated for 45 min before collecting 15-min fractions to assay resting release of vasopressin and release evoked by electrical stimulation of the pituitary stalk (evoked release = average vasopressin release during 15 min stimulation sample + 15 min post-stimulation period, see Fig. 1). Mean values ± s.e.m. for number of experiments (n) indicated.

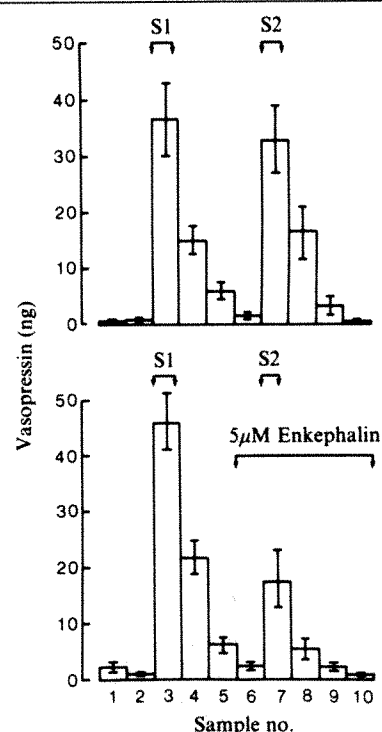


Fig. 1 Release of immunoreactive vasopressin from rat pituitary *in vitro* and inhibition by a stable enkephalin analogue. Rat pituitary pars nervosa/pars intermedia fragments were attached by the pituitary stalk to suction electrodes and incubated in Krebs-bicarbonate medium for 60 min before starting to collect sequential 15-min samples. During the experiment each gland was subjected to two periods of electrical stimulation (S1 and S2) through the pituitary stalk (30 V, 0.2 ms duration, 30 Hz in 10-s trains with 10-s intervals between trains for a total of 10 min). In 14 control experiments (a) the release of the vasopressin evoked during S2 was similar to that observed during S1. Addition of a stable enkephalin analogue (D-Ala², D-Leu⁵-enkephalin = 5 µM ENKEPHALIN) 15 min before S2 (b, eight experiments). Vasopressin was measured by radioimmunoassay and results are expressed as ng Arg⁸-vasopressin equivalents; each value is the mean ± s.e.m. for the number of experiments indicated.

15-min intervals and vasopressin was measured by a radioimmunoassay procedure, using ¹²⁵I-vasopressin (NEN), a rabbit antibody to vasopressin (Calbiochem-Behring) and synthetic Arg-vasopressin as standard. The immunoassay had a sensitivity limit of 0.2-0.4 pg per sample and there was no detectable cross-reactivity with oxytocin in amounts up to 200 ng. None of the test substances used had any effects on the vasopressin radioimmunoassay. Incubation samples were neutralised (10 µl 0.2 M HCl) and stored frozen; they were diluted at least 10-fold with assay buffer before assay. As the spontaneous release of vasopressin was initially high, but subsequently declined to low levels, the first period of electrical stimulation was carried out after a preincubation period of 60 min. Phasic rather than continuous stimulation was chosen as this more closely mimics the natural firing pattern of the vasopressin neurones, and leads to an enhanced release of vasopressin *in vitro*⁴. In these conditions the resting release of vasopressin in 14 control experiments was 0.6 ± 0.13 ng Arg⁸-vasopressin equivalent in 15 min, corresponding to a spontaneous release rate of approximately 0.004% of total tissue content per min (tissue content at the end of experiment = 1,072.5 ± 137.0 ng vasopressin per gland, mean ± s.e.m., n = 14). Phasic electrical stimulation of the pituitary stalk (S1) led to an increase of more than 60-fold in vasopressin release, and a total of 51.2 ± 8.94 ng vasopressin (mean ± s.e.m., n = 14) was recovered in the stimulation sample and in the sample collected in the 15-min period after stimulation (Fig. 1). A second period of electrical stimulation (S2) 45 min after the first elicited almost the same release of vasopressin (49.1 ± 10.59 ng) as the first. In subsequent experiments

therefore, test agents were introduced 15 min before the second period of stimulation, and results were expressed as the ratio of vasopressin released during the two stimulation periods (S2/S1).

Control experiments confirmed²⁻⁴ that the stimulation-evoked release of vasopressin was completely abolished by the addition of tetrodotoxin ($1 \mu\text{g ml}^{-1}$) or by removal of calcium ions from the incubation medium (Table 1). Addition of the stable enkephalin analogue D-Ala²,D-Leu⁵-enkephalin (DADLE)⁵ ($5 \mu\text{M}$) before the second stimulation period led to more than a 70% inhibition of the stimulus-evoked release of vasopressin (Fig. 1, Table 2), without any significant change in the spontaneous efflux of vasopressin. Similar inhibitory effects on the stimulation-evoked release of vasopressin were seen after addition of morphine ($10 \mu\text{M}$) or β -endorphin ($2 \mu\text{M}$). The inhibitory effect of DADLE was completely reversed by simultaneous addition of the opiate antagonist drug naloxone ($1 \mu\text{M}$) (Table 2). Naloxone alone, however, had no significant effect on the spontaneous or evoked release of vasopressin; γ -aminobutyric acid (GABA) ($50 \mu\text{M}$) and somatostatin ($1 \mu\text{g ml}^{-1}$) were also without effect (Table 2).

The results show that activation of opiate receptors in the rat neurohypophysis can inhibit the stimulus-evoked release of vasopressin from neurosecretory nerve terminals. These findings suggest that opiate receptors may exist on the terminals of vasopressin neurones in the pars nervosa, which possesses a high density of opiate binding sites⁶. The effects of opiates are selective, as two other biologically active compounds that occur in and can be released from the pars nervosa, GABA⁷ and somatostatin⁸, had no effects on vasopressin release. The present findings are at first sight difficult to reconcile with several previous reports which suggest that the administration of opiates or endorphins *in vivo* leads to an enhanced release of vasopressin from the pituitary⁹⁻¹². However, this general conclusion has been questioned¹³, and recent studies have reported that systemically or centrally administered opiates and endorphins can lead to a reduction in vasopressin immunoreactivity in rat plasma^{14,15}. In addition to the inhibitory effects on vasopressin release from neurohypophysis reported here, opiates may act centrally to inhibit the firing of vasopressin neurones (ref. 16 and G. Clarke and P. Wood, personal communication). Furthermore, the present results are complementary to those of Clarke *et al.*¹⁷, who recently reported that intraventricularly administered morphine led to a suppression of oxytocin release from rat pituitary, and suggested the existence of opiate receptors on oxytocin-containing nerve terminals in the neurohypophysis. Our findings confirm a previous report that β -endorphin failed to affect the spontaneous release of vasopressin from rat pars nervosa *in vitro*¹², although these authors did not examine the

effects of endorphin on the stimulus-evoked release of vasopressin. The concept that opiate receptors may exert an inhibitory influence on the release of a biologically active substance from nerve terminals agrees with other studies which have shown that opiate receptors in other parts of the nervous system control the release of acetylcholine¹⁸, noradrenaline¹⁸ and substance P¹⁹. The possible physiological function of such a control mechanism in the neurohypophysis remains to be defined.

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Multiple opiate receptor sites on primary afferent fibres

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In addition to their actions at supraspinal sites, opiates can act directly at the spinal cord level to produce analgesia¹⁻³. Opiate receptors and enkephalins are found in abundance in the dorsal horn of the spinal cord, in the region of termination of small-diameter primary afferents⁴⁻⁶. Furthermore, there is evidence that exogenously administered opiates or endogenous enkephalins act both on postsynaptic receptors in dorsal horn and presynaptically to block transmitter release from nociceptive primary afferent terminals⁷⁻¹⁰. The possibility of these two distinct sites of opiate action in dorsal horn is of particular interest in view of recent evidence for the existence of multiple opiate receptors¹¹⁻¹³. On the basis of differences in the rank orders of potency of opiate alkaloids and opioid peptides observed in different tissues, Lord *et al.*¹⁴ have proposed two categories of opiate receptor, μ and δ , and these can be differentiated in binding studies, provided that selective radioligands are used in the appropriate experimental conditions¹⁵⁻¹⁸. Here we have used the selective radioligands ³H-morphine (μ sites) and ³H-D-Ala²,D-Leu⁵-enkephalin (δ sites) to examine directly in rat the distribution and binding characteristics of opiate receptors on dorsal root and in various regions of the adjacent spinal cord. Primary afferent tissue (dorsal root) and dorsal horn were found to contain μ and δ opiate binding sites with a relatively high proportion of μ sites. Partial destruction of small-diameter primary afferents after cutting the sciatic nerve led to a significant reduction in both μ and δ binding sites on dorsal roots, suggesting that both types of opiate receptors may exist on small-diameter primary afferents.

Table 2 Effect of opiates and other substances on stimulation-evoked release of vasopressin from rat pars nervosa

	n	S2/S1 (mean \pm s.e.m.)
Control	14	0.99 \pm 0.12
D-Ala ² ,D-Leu ⁵ -enkephalin ($5 \mu\text{M}$)	8	0.28 \pm 0.08*
Morphine sulphate ($10 \mu\text{M}$)	5	0.28 \pm 0.10*
β -Endorphin ($2 \mu\text{M}$)	3	0.44 \pm 0.14*
D-Ala ² ,D-Leu ⁵ -enkephalin ($5 \mu\text{M}$) + naloxone ($1 \mu\text{M}$)	7	1.06 \pm 0.26
Naloxone ($1 \mu\text{M}$)	5	0.97 \pm 0.24
Somatostatin ($1 \mu\text{g ml}^{-1}$)	3	0.98 \pm 0.18
GABA ($50 \mu\text{M}$)	3	1.14 \pm 0.29

After a control period of electrical stimulation (S1), test substances were added to the incubation medium 15 min before a second period of electrical stimulation (S2). Results are expressed as the ratio S2/S1, representing the total amounts of vasopressin released (during the stimulation period and in post-stimulation sample) by the two stimulation periods. Values are means \pm s.e.m. for the number of experiments indicated (n).

* $P < 0.01$ compared with control.

Using a modification of the approach described by Chang and Cuatrecasas¹⁸, commercially available high-specific activity tritium-labelled morphine and D-Ala²,D-Leu⁵-enkephalin at low concentrations were chosen as selective radioligands for μ and δ opiate binding sites, respectively^{17,18}. As described by Lord *et al.*¹⁴, μ receptors are characterised by their high affinity for morphine and related alkaloids and for the antagonist drug naloxone, whereas δ receptors preferentially recognise enkephalins and are relatively resistant to naloxone. Preliminary experiments were carried out using rat corpus striatum, a region of the central nervous (CNS) system known to contain a high density of both μ and δ binding sites¹⁷. There was a considerable amount of specific binding (defined by the use of 1 μ M levorphanol) of both radioligands in membrane preparations from striatum; at low concentrations of each ligand (≤ 1 nM) specific binding accounted for 80–90% of total binding. Saturation analyses over the concentration range of 0.1–15.0 nM for ³H-D-Ala²,D-Leu⁵-enkephalin indicated the presence of a single binding component on Scatchard plots, with an apparent K_d of 2.4 nM (Table 1). Similar analyses for ³H-morphine suggest the existence of both low- and high-affinity binding components, but over a restricted range of low concentrations of ligand (0.2–1.5 nM) a single high-affinity component predominated, with an apparent K_d of 1.2 nM. The potency of various opiates, opiate antagonists and opioid peptides in competing for ³H-morphine or ³H-D-Ala²,D-Leu⁵-enkephalin binding was determined, using the approach described by Terenius and Wahlström¹⁶ in which a low concentration of radioligand (0.5 nM) was combined with the same concentration of the second unlabelled ligand to

Table 1 Properties of ³H-morphine and ³H-D-Ala²,D-Leu⁵-enkephalin binding in rat striatum and spinal cord

	Striatum		Spinal cord	
	³ H-morphine	³ H-D-Ala, D-Leu-enkephalin	³ H-morphine	³ H-D-Ala, D-Leu-enkephalin
Apparent K_d (nM \pm SEM)	1.2 \pm 0.08	2.4 \pm 0.18	1.0 \pm 0.28	3.9 \pm 0.64
	IC ₅₀ values (nM)			
D-Ala ² ,D-Leu ⁵ - enkephalin	15.0	3.8	16.0	7.6
Met ⁵ -enkephalin	4.0	2.6	—	—
Leu ⁵ -enkephalin	5.2	1.0	—	—
Levorphanol	1.0	11.0	0.9	2.1
Morphine	2.5	240.0	3.0	68.0
Naloxone	1.8	34.0	3.5	14.0
Diprenorphine	0.4	0.16	—	—
Ethylketocyclazocine	3.4	7.4	—	—

Corpus striatum or whole spinal cord were dissected from adult male Sprague-Dawley rats and homogenised in 50 vol ice-cold 50 mM Tris-HCl buffer, pH 7.4 (Polytron, setting 7, 15 s). The homogenate was centrifuged (4°C, 50,000g, 15 min) and resuspended in 50 vol Tris buffer (Polytron, setting 7, 15 s). After incubation for 45 min at 37°C, to digest endogenous enkephalins, membrane fragments were collected by centrifugation and suspended in ice-cold Tris buffer at a final concentration (in terms of original tissue wet weight) of 10 mg ml⁻¹ (striatum) or 20 mg ml⁻¹ (spinal cord). Samples (1 ml) of the membrane suspension were incubated with ³H-morphine ([1(n)-³H]morphine; specific activity 27 Ci mmol⁻¹; Radiochemical Centre) or ³H-D-Ala²,D-Leu⁵-enkephalin (D-Ala²,D-Leu⁵-[tyrosyl-3,5-³H]enkephalin; specific activity 48 Ci mmol⁻¹; Radiochemical Centre) for 20 min at 30°C. The labelled membranes were collected by rapid filtration on Whatman GF/B glass fibre filter disks, and washed with 2 \times 5 ml of Tris buffer. After soaking the filters for at least 2 h in 4 ml ethoxyethanol, radioactivity was measured by liquid scintillation counting after addition of 10 ml scintillation fluid. Nonspecific binding was defined by parallel incubations in the presence of 1 μ M levorphanol. Incubations and non-specific blanks were always carried out in triplicate. The protein content of individual membrane preparations was determined by the method of Lowry *et al.* To avoid losses of ³H-enkephalin due to adsorption to glassware and pipettes the stock solution of the labelled peptide was diluted in plastic tubes with Tris buffer containing bovine serum albumin (Sigma; crystalline grade) 0.1 mg ml⁻¹ in preparing a stock solution; this was further diluted 1:500 (v/v) in the final incubations. IC₅₀ values were determined at 0.5 nM radioligand concentration as illustrated in Fig. 1. K_d values were determined by Scatchard analysis of specific binding measured in triplicate at 4–6 ligand concentrations in the concentration range 0.1–15.0 nM for ³H-D-Ala²,D-Leu⁵-enkephalin, and 0.2–1.5 nM for ³H-morphine.

Table 2 Distribution of opiate binding sites in rat spinal cord, striatum and spinal roots

	Specific binding (fmol per mg protein)		
	³ H-morphine	³ H-D-Ala, D-Leu-enkephalin	Binding ratio
Dorsal root	8.9 \pm 0.45(7)	2.7 \pm 0.22(6)	3.2 \pm 0.22(6)
Dorsal horn	8.1 \pm 0.66(4)	4.9 \pm 0.79(4)	1.7 \pm 0.28(4)
Ventral horn	3.6 \pm 0.56(4)	3.6 \pm 0.20(4)	1.0 \pm 0.11(4)
Ventral root	2.2 \pm 0.31(3)	<1.0(3)	—
Sciatic nerve	<1.0(5)	<1.0(5)	—
Corpus striatum	24.0 \pm 2.54(7)	44.2 \pm 2.69(7)	0.5 \pm 0.04

Spinal tissues were removed following laminectomy in stunned decapitated rats. Roots were dissected from L4–L6 ganglia to cord entry, sciatic nerve from notch to knee. Each cord half was divided at the level of the canal into dorsal and ventral parts. Each measurement included pooled tissue from 6 to 10 adult male rats (250–250 mg tissue). All measurements were made at 0.5 nM concentrations of radioligand. Specific binding, defined using 1 μ M levorphanol, was 40–60% of total for ³H-morphine and 20–45% for ³H-D-Ala²,D-Leu⁵-enkephalin. In some cases the specific binding of radioligands was less than 1 fmol per mg protein and was considered to be below the limit for accurate measurement (<20% specific binding). Using the Student's *t*-test, the amount of ³H-morphine binding in ventral horn differed significantly from that in dorsal horn ($P < 0.005$) and dorsal root ($P < 0.001$). For the binding ratios, dorsal horn differed significantly from dorsal root ($P < 0.01$) and ventral horn ($P < 0.01$). Other comparisons did not show significant differences. Statistical comparison with ventral root and sciatic nerve were not made. Values are means \pm s.e.m. for the number of experiments indicated in brackets. The binding ratio represents binding of each radioligand at a single concentration (0.5 nM) and gives a relative indication of the proportions of μ and δ binding sites.

ensure that drug potencies were compared in conditions of similar basal receptor occupancy.

The results obtained with the various drugs tested (Fig. 1, Table 1) were similar to those reported by Chang and Cuatrecasas¹⁸, who used ³H-dihydromorphine or ³H-naloxone and ¹²⁵I-D-Ala²,D-Leu⁵-enkephalin. Provided the tritium-labelled compounds were used at concentrations below their K_d values (0.5 nM), they seemed to serve as selective ligands for μ and δ opiate receptors, respectively. Morphine itself was the best discriminating drug, as it was ~100 times more potent in competing for the binding of ³H-morphine (μ sites) than for ³H-D-Ala²,D-Leu⁵-enkephalin (δ sites, Fig. 1). Naloxone was ~20 times more potent on μ than on δ sites, and nonradioactive D-Ala²,D-Leu⁵-enkephalin, Met-enkephalin and Leu-enkephalin were more potent on δ than on μ sites. Because neither ³H-morphine nor ³H-D-Ala²,D-Leu⁵-enkephalin are completely selective ligands for μ and δ sites, saturation analysis cannot provide accurate values for the absolute numbers of μ and δ sites in particular regions of the CNS. However, the use of these radioligands at low concentrations provides a useful index of the ratio of μ to δ sites. The present results suggest a preponderance of enkephalin (δ) receptors over morphine (μ) receptors in corpus striatum.

By using homogenates of whole rat spinal cord, binding sites for both radioligands could be detected with K_d values and inhibitor sensitivities similar to those observed in striatum (Table 1). Although the density of binding sites in spinal cord was much lower than in striatum, there was significant binding of both the peptide (δ) and alkaloid (μ) agonists on pooled samples of lumbosacral dorsal roots and in dorsal and ventral horn. Binding sites for either ligand were barely detectable on ventral root or peripheral nerve (Table 2). Compared with striatum, where δ binding predominated (with a μ/δ ratio of about 0.5), there was relatively more μ binding in dorsal root and dorsal horn, with μ/δ binding ratios of 3.2 and 1.8, respectively (Table 2). To determine whether the ³H-enkephalin (δ) binding in dorsal roots represented a possible cross-reactivity of this ligand with μ receptors, displacement studies were carried out with nonradioactive morphine. Addition of 25 nM nonradioactive morphine caused an 85% inhibition of ³H-morphine binding in

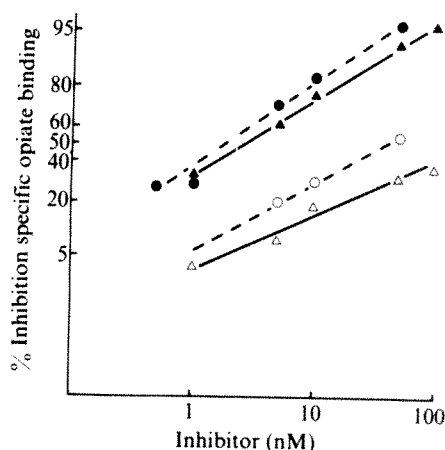


Fig. 1 Determination of IC_{50} values for morphine (triangles) and naloxone (circles) as inhibitors of specific binding of 3H -morphine (solid circles and triangles) and 3H -D-Ala,D-Leu-enkephalin (open circles and triangles, each at 0.5 nM) in rat striatal membrane preparation. Each point represents the mean of triplicate determinations. All tubes for 3H -morphine binding measurements also contained 0.5 nM nonradioactive D-Ala,D-Leu-enkephalin, and tubes for 3H -enkephalin binding contained 0.5 nM nonradioactive morphine to ensure similar basal receptor occupancy¹⁶. Ordinate: per cent inhibition of specific 3H -labelled radioligand binding (defined by 1 μM levorphanol) probit scale; abscissa: log concentration of nonradioactive morphine or naloxone.

dorsal root samples, but less than a 10% inhibition of 3H -D-Ala,D-Leu-enkephalin binding ($n=2$). Because of the small amounts of dorsal root tissue obtainable, it was not possible to carry out detailed saturation analyses or complete drug displacement curves. However, the results clearly demonstrate that both types of opiate site are present on primary afferent fibres. Furthermore, the observation that binding ratios for the two types of agonist differ significantly on dorsal root and dorsal horn supports the idea that there are separate pre- and post-synaptic populations of opiate receptor, with a relatively greater proportion of δ -type receptors on postsynaptic elements in the dorsal horn.

To determine whether the opiate binding sites are present on small-diameter primary afferent fibres, further experiments were carried out after cutting the sciatic nerve. There is evidence that this procedure leads to a loss predominantly of small-diameter afferent fibres in the dorsal roots¹⁹⁻²¹. In three groups of 10 rats, the sciatic nerve was cut 1 month before dissection of the L5 and L6 dorsal roots, which were pooled to prepare membranes for ligand binding studies. The results showed a significant reduction in both μ and δ binding sites on the dorsal roots after sciatic nerve section (Table 3).

The present results provide the first direct evidence that primary afferents have significant numbers of opiate receptor sites on their central processes. This conclusion is consistent with previous findings of a depletion of opiate binding sites in dorsal horn following damage to primary afferents^{22,23}. Autoradiographic evidence has also shown that the central processes of vagus nerve afferents, which are predominantly unmyelinated, bear opiate receptors²⁴. Furthermore, administration of capsaicin to neonatal rats causes a relatively selective loss of small-diameter primary afferents²⁵ and this procedure leads to a

reduction of opiate receptors in dorsal horn^{26,27}. These findings are consistent with neurophysiological evidence that opiates can act directly on the terminals of small-diameter primary afferent fibres^{28,29} and exert a selective inhibitory effect on nociceptive input to dorsal horn neurones^{9,10}, adding further support to the hypothesis that opiates may act presynaptically to block pain transmission at the spinal cord level.

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Changes in composition and activity of microtubule-associated proteins during brain development

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The onset of neuronal differentiation is characterised by intensive neurite growth; because microtubule formation is strictly required during this process¹⁻³, *in vitro* assembly of the tubulin present in the rat brain has been studied at different stages of development⁴: the rate of assembly is very slow in the early stages and increases progressively with age from birth until adulthood. Other data⁴ also suggested that the limiting factor in the young brain is the amount or activity of one or several of the minor components which co-polymerise into microtubules with tubulin. We show here that both the composition and the activity of the microtubule-associated proteins change during the time course of rat brain development.

Rat brain supernatants were prepared at two stages of post-natal development, 3 days after birth (that is, at the beginning of the critical period of development in this species) and at day 35 (near adulthood). The tubulin present in both supernatants was allowed to assemble *in vitro* in the conditions described by

Table 3 Reduction of μ and δ binding in dorsal roots 1 month following sciatic cut

	Specific binding (fmol per mg protein)	
	3H -morphine (μ)	3H -D-Ala,D-Leu-enkephalin (δ)
Normal control (Table 2)	8.9 \pm 0.45(7)	2.7 \pm 0.22(6)
Sciatic cut	5.9 \pm 1.18(3)	1.3 \pm 0.05(3)
% Reduction	-34%	-51%

The sciatic nerve was cut 30 days before removal of the dorsal roots (L5, L6). The cut side significantly differed from control ($P < 0.05$ for μ , $P < 0.005$ for δ).

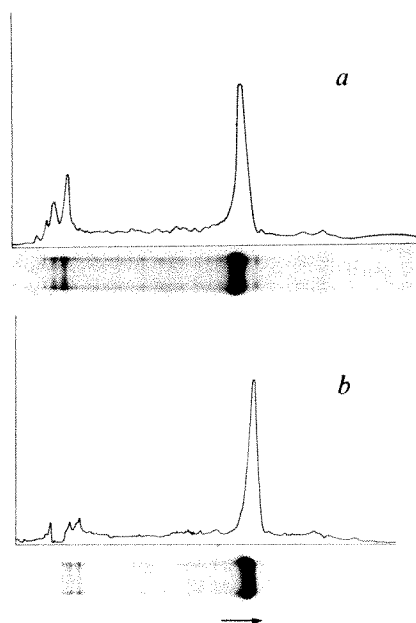


Fig. 1 Analysis by SDS gel electrophoresis of microtubule proteins prepared from adult (a) and 3-d-old (b) rat brain. Microtubules were obtained by the procedure described by Shelanski *et al.*⁵ slightly modified⁶. The microtubule pellets were depolymerised by cold in the presence of NaCl 0.75 M. Samples of these preparations were made up with 1% SDS and 1% dithiothreitol and then heated to 100°C for 5 min. Aliquots of these samples containing 11 µg of microtubule proteins were electrophoresed according to the method of Weber and Osborn⁷ on a slab gel with a 4–15% gradient of polyacrylamide. The gels stained with Coomassie blue are represented for the two preparations of microtubule proteins.

Shelanski *et al.*⁵ slightly modified⁶. At the end of the incubation period the microtubules were pelleted by centrifugation, washed and analysed by polyacrylamide gel electrophoresis in the conditions described by Weber and Osborn⁷. Fig 1a and b, show that, in addition to tubulin, several minor components are present in both 'young' and 'adult' preparations. Among these associated proteins one group with high molecular weight (~300,000) contains two components similar to MAP₁ and MAP₂ (or HMW₁ and HMW₂) first described by Murphy and Borisov^{8,9}. The young preparation contains both MAP₁ and MAP₂; several analyses show that the percentage of these entities does not differ from that measured in adult microtubules (MAP₁ + MAP₂/tubulin = 16% in the young preparation and 17.5% in the adult one). Another group of MAPs, not well separated from tubulin and similar to the τ protein complex first isolated by Weingarten *et al.*¹⁰, is seen in the microtubules of both ages. The τ complex is thermostable¹⁰, and so, to study better this group of microtubule-associated proteins at both ages, purification was achieved as previously described¹¹ by thermic treatment of the *in vitro* assembled microtubules. Tubulin and MAP₁ were eliminated during this treatment and the other microtubule-associated proteins could be analysed at a much higher concentration by polyacrylamide gel electrophoresis. Figure 2a, which represents the analysis of the adult associated proteins, shows the presence of MAP₂ and the τ complex with its characteristic four closely spaced bands. With the young preparation MAP₂ is clearly present (Fig. 2b), but the pattern at the level of the τ complex is completely different: only two bands are present and none of them seem to be identical to any of the four bands present in the adult preparation. Identical results were found in four different experiments; moreover, after a second cycle of assembly the same protein pattern was found at the level of the τ complex, only two bands for the young preparation, with none of them being similar to any of the four closely spaced bands of the adult one. In addition, a control experiment was

carried out in the presence, throughout the procedure, of a protease inhibitor (phenylmethylsulphonyl fluoride) to rule out the possibility that the changes seen at the level of the τ complex are due to proteolytic breakdown of this or other (MAP₁, MAP₂)¹² fractions in the young preparation. Polyacrylamide gel electrophoresis revealed no marked differences in the protein patterns.

The activity of young and adult microtubule-associated proteins preparations in promoting tubulin assembly was also assayed. Pure adult tubulin was prepared by phosphocellulose chromatography^{11,13}; as previously shown, such tubulin does not assemble *in vitro* into microtubules even at high concentrations^{8,10,11,14,15}. A constant amount of pure tubulin was then incubated at 37°C in the presence of increasing concentrations of both young and adult associated proteins obtained by thermic treatment; Fig. 3a shows the time course of assembly. Clearly, the activity of the young microtubule-associated proteins was much lower than that of the adult one. Fig. 3b represents the relationship between initial rates of assembly and the concentration of young or adult microtubule-associated

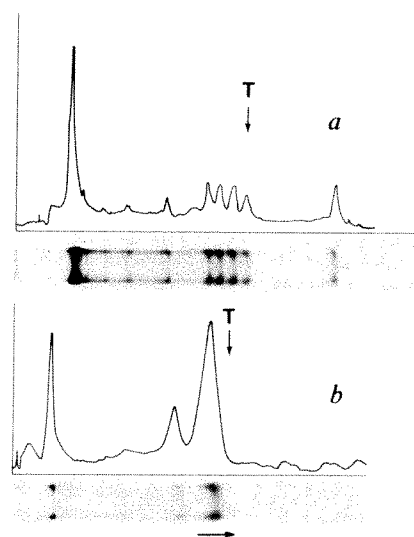


Fig. 2 Analysis by SDS gel electrophoresis of thermostable microtubule-associated proteins (or MAPs) prepared from adult (a) and 3-d-old (b) rat brain. Thermostable MAPs were prepared by a thermic denaturation procedure previously described¹². Samples of MAPs containing 7 µg for 3-d-old rat brain preparation and 11 µg for adult rat brain preparation were electrophoresed in the conditions described in Fig 1 legend. The gels stained with Coomassie blue are represented for the two kinds of MAP preparations. T, Position of tubulin.

protein preparations. It can be seen that (1) the rate of assembly increases linearly with both preparations and remains always much lower for the young one, and (2) the minimal concentration of microtubule-associated proteins at which assembly may be observed (critical concentration¹⁶) is higher with the young than the adult preparation. Similarly, we have previously shown that the critical concentration of tubulin is higher with young crude brain supernatants than with adult ones⁴.

Because young microtubule-associated proteins contain MAP₂, which is active¹⁷ in promoting *in vitro* microtubule assembly, but not the normal τ fraction distribution, one may speculate that the activity present at the early stage is due to MAP₂ only. Conversely, one or both of the two peaks migrating in the τ region might have some activity. Purification of the slow and fast components present in the young preparation is in progress so that their activity may be tested separately.

From the data reported here, a model may be proposed to explain the role of increasing neurotubule assembly during brain development. Large pools of tubulin would be synthesised before the building up of the neuronal network^{18,19}; some or all

of this tubulin would be used at earlier stages of development to allow the formation of the mitotic spindle and therefore proliferation of the undifferentiated nerve cells. At the beginning of the differentiation phase, specific proteins with polymerising activity would appear in increasing amounts, thereby allowing fast microtubule assembly and intensive neurite growth. Thus, the transition between cell division and cell differentiation might be controlled, at least in part, by an increase in the polymerising activity or by qualitative changes in the factors required for

either pathway or both. Although further work is needed to decide on the requirements for microtubule assembly before nerve cell differentiation, our data support the idea that the appearance of specific factors is responsible for the increase in the rates of microtubule assembly during the critical period of brain development—during (and for) intensive neurite growth.

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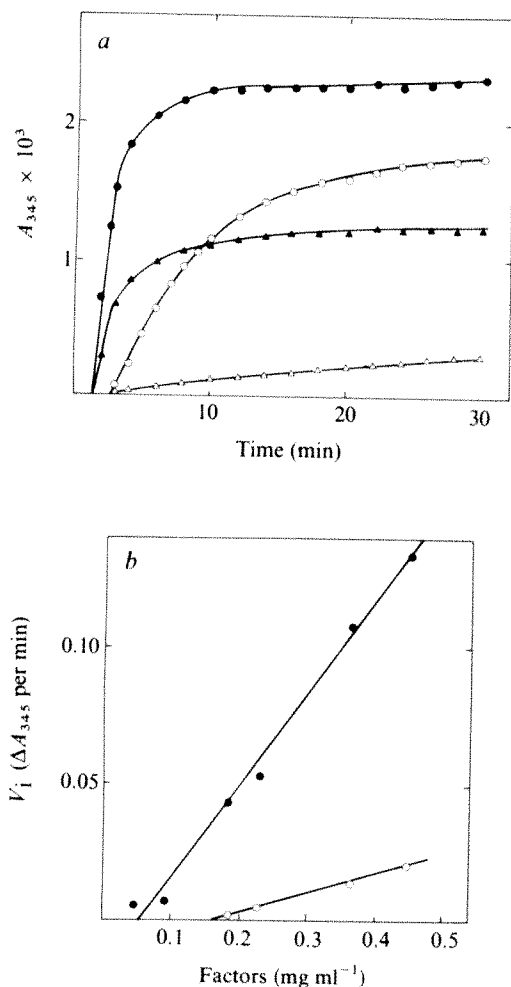


Fig. 3 Polymerisation-promoting activity of adult and 3-d-old rat brain thermostable microtubule-associated proteins on purified tubulin. Purified tubulin was obtained by a procedure previously described by Weingarten *et al.*¹⁰ but slightly modified: activated phosphocellulose was resuspended in the assembly buffer containing (in mM) morpholinethane sulphonate 100, MgCl_2 0.5, EGTA 1, EDTA 0.1, GTP 1 and 2-mercaptoethanol 1, pH 6.4. Tubulin was eluted from the column with the same buffer. The purity of the tubulin was controlled by SDS gel electrophoresis. The polymerisation-promoting activity of the two kinds of MAPs on purified tubulin were followed by the turbidimetric method described by Gaskin *et al.*¹⁶. Turbidimetry measurements (345 nm) during tubulin polymerisation, at 37 °C, in the assembly buffer previously described, were carried out using a Carl Zeiss PM 6KS spectrophotometer with an automatic thermostated four-sample changer. *a*, Time course of tubulin polymerisation in the presence of the two preparations of thermostable microtubule-associated proteins. Pure tubulin (0.95 mg ml^{-1}) was incubated in the presence of two concentrations of thermostable MAPs: 'adult' MAPs (\blacktriangle , $181 \mu\text{g ml}^{-1}$; \bullet , $452 \mu\text{g ml}^{-1}$) and '3-d-old' MAPs (\triangle , $181 \mu\text{g ml}^{-1}$; \circ , $452 \mu\text{g ml}^{-1}$). *b*, Relationship between initial rates of tubulin polymerisation and thermostable MAPs concentration. Initial rates of tubulin polymerisation (ΔA_{345} per min) were measured with increasing concentrations of adult MAPs (\bullet) and 3-d-old MAPs (\circ) and a constant tubulin concentration (0.95 mg ml^{-1}).

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In vivo release of acetylcholinesterase in cat substantia nigra and caudate nucleus

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Acetylcholinesterase (AChE), which is known to inactivate acetylcholine (ACh), is present in great abundance in the substantia nigra, although ACh levels and choline acetylase activity in this region are relatively low¹. Nigral dopaminergic cell bodies and their dendrites also contain AChE^{2–4}. The functional significance of this enzyme in nigro-striatal dopaminergic neurones has been questioned^{1,4,5}. Earlier studies demonstrated an evoked release of AChE from unidentified central neurones into cerebrospinal fluid (CSF) in cats⁶, rabbits^{7,8} and dogs⁹. Later experiments have provided indirect evidence that the substantia nigra may contribute to a substantial amount of AChE detected: a unilateral nigral lesion in rabbits reduced AChE levels in the CSF, whereas electrical stimulation of the substantia nigra induced the opposite effect¹⁰. To investigate the possible release of AChE from dopaminergic dendrites and terminals we measured the *in vivo* release of this enzyme from the substantiae nigrae and caudate nuclei of cats implanted with four push-pull cannulae and compared it with that of dopamine (DA). DA is released from dendrites in the substantia nigra^{11,12} as well as nerve terminals in the caudate nucleus. Spontaneous AChE release was observed in the substantia nigra and in the caudate nucleus. Moreover, the application of potassium (30 mM) in one substantia nigra increased the local release of AChE. This was accompanied by remote changes in the enzyme release from the other three structures which differed from that seen for DA. The different patterns of responses observed for AChE and DA suggest that AChE may also originate from other neurones in both the substantia nigra and the caudate nucleus.

Push-pull cannulae were acutely implanted in the two caudate nuclei and substantia nigrae of halothane-anaesthetised cats as described previously¹¹. The four structures were superfused with artificial CSF at a rate of 1.2 ml h⁻¹ and the activity of AChE^{13,14}, nonspecific cholinesterase^{13,14}, and occasionally lactate dehydrogenase¹⁵, were estimated in successive 10-min fractions. In a second series of experiments the release of ³H-DA continuously synthesised from L-[3, 5-³H]-tyrosine (50 Ci mmol⁻¹, 50 μ Ci ml⁻¹) was estimated in the four structures¹⁶. In all cases, the stereotaxic placement of the tip of each push-pull cannula was verified histologically.

A spontaneous release of AChE, which remained constant throughout the experiment, was observed both in the substantia nigrae and in the caudate nuclei. The amount of AChE released was similar in both structures (Fig. 1). Nonspecific cholinesterase was also observed in superfusates, the levels being slightly higher than those of AChE in the substantia nigrae and in the caudate nuclei (Fig. 2).

The addition of potassium chloride (30 mM) for 10 min to the artificial CSF in the left substantia nigra markedly increased the local release of AChE, the maximal change being seen during the treatment. Surprisingly, this effect was accompanied by changes in AChE release in the three other structures. Whereas AChE release was also increased in the contralateral caudate nucleus, it was decreased in the ipsilateral caudate nucleus and the contralateral substantia nigra (Fig. 1). These distal changes were more sustained than those observed in the left substantia

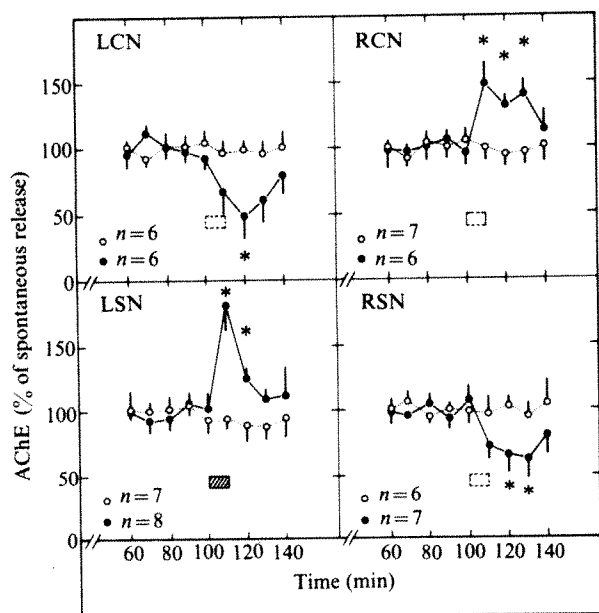


Fig. 1 Effects of the unilateral nigral application of potassium (30 mM) on the release of acetylcholinesterase (AChE) in both substantia nigrae and caudate nuclei. Four push-pull cannulae were simultaneously implanted in the left (LCN) and the right (RCN) caudate nuclei and in the left (LSN) and the right (RSN) substantia nigrae in anaesthetised cats. The four structures were superfused with an artificial CSF (1.2 ml h⁻¹) and AChE was estimated in 10-min successive superfusate fractions 60 min after the beginning of the superfusions. Potassium chloride (30 mM) was applied for 10 min in the LSN (hatched box with solid sides). The empty boxes with dotted sides represent the time during which potassium chloride (30 mM) was applied in the LSN. In each animal, and for each cannula, AChE in each successive fraction was expressed as a percentage of an average spontaneous release calculated from the five fractions collected (1.5 \pm 0.4, 1.8 \pm 0.5 and 1.7 \pm 0.3, 1.6 \pm 0.5 mU ml⁻¹ in the left and right substantia nigra and caudate nucleus respectively). Data are the mean \pm s.e.m. of results obtained with treated animals (●). $P < 0.05$ when compared with corresponding control values obtained in untreated animals (○).

nigra. In contrast, the release of nonspecific cholinesterase was not affected in the left substantia nigra or the other three structures (Fig. 2). As cat plasma contains a substantial amount of nonspecific cholinesterase (269 \pm 47 mU ml⁻¹, $n = 5$) as well as AChE (254 \pm 44 mU ml⁻¹, $n = 5$), the unchanged levels of nonspecific cholinesterase in superfusates would exclude a plasma origin for the observed changes in the release of AChE. Furthermore, the local release of AChE induced by potassium cannot be attributed to gross cell damage as the activity of lactate dehydrogenase (a cytoplasmic enzyme) in the superfusates remained unchanged during potassium application (before, 1.5 \pm 0.6 mU ml⁻¹; after, 1.5 \pm 0.6 mU ml⁻¹; $n = 4$). Therefore, the release of AChE is a specific process.

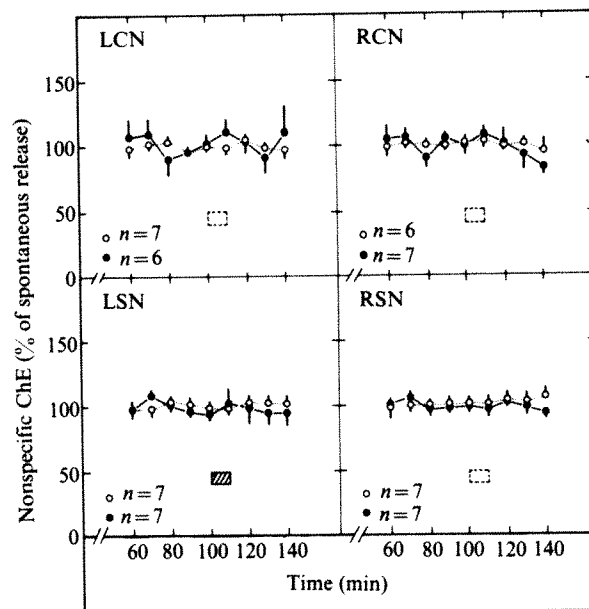


Fig. 2 Lack of effect of the unilateral nigral application of potassium (30 mM) on the release of nonspecific cholinesterase (ChE) in both substantia nigrae and caudate nuclei. During the experiments described in Fig. 1, we also measured nonspecific ChE in 10-min superfusate fractions and expressed the results as described in Fig. 1. The mean spontaneous release of nonspecific ChE in the left and right substantia nigra and in caudate nucleus were 2.3 \pm 0.5, 2.7 \pm 0.7 and 2.4 \pm 0.5, 2.6 \pm 0.6 mU ml⁻¹, respectively. Data are the mean \pm s.e.m. of results obtained with control (○) and treated cats (●). Boxes as in Fig. 1.

Studies on peripheral nerves and innervated tissue have indicated that AChE can be released through a process dependent on nerve activity^{17,18}. The distal changes in AChE release observed in the present study suggest that this phenomenon also occurs in the central nervous system (CNS). There is, therefore, little doubt that the release of AChE in the substantia nigrae and caudate nuclei has a physiological significance.

If AChE originates from nigro-striatal dopaminergic neurones, the simplest hypothesis is that AChE should behave as does DA released from dendrites and nerve terminals in the substantia nigra and caudate nucleus respectively. We thus measured the release of newly synthesised ³H-DA in the four structures. Confirming previous results, the unilateral nigral application of potassium (30 mM) enhanced the release of ³H-DA in both caudate nuclei, the effect being slightly less pronounced in the contralateral structure¹⁹. Moreover, potassium increased the release of ³H-DA not only locally in the left substantia nigra but also, surprisingly, in the right substantia nigra. Therefore, the nigral application of potassium induced opposite effects on AChE and ³H-DA release in the ipsilateral

caudate nucleus and the contralateral substantia nigra. These findings do not exclude the possibility of a release of AChE from dopaminergic dendrites and nerve terminals. However, in this case, the messages reaching the ipsilateral caudate nucleus or the contralateral substantia nigra and responsible for the release of AChE and DA would have to be mediated by different pathways, or the two compounds would have to display a differential response to the same signal. Another hypothesis is that AChE originates additionally or exclusively from non-dopaminergic neurones.

Although histochemical and biochemical studies have clearly indicated that a high proportion of AChE is present in dopaminergic cell bodies and their dendrites, nigral AChE also has another location. As shown in the rat substantia nigra, 6-hydroxydopamine lesions, which reduced tyrosine hydroxylase activity by at least 90%, decreased that of AChE by merely 43% (ref. 4). Furthermore, nigral AChE was only reduced by 44% after a local lesion with kainic acid, a neurotoxin which completely destroyed the dopaminergic neurones and is also known to induce the degeneration of other nigral neurones²⁰. AChE not contained in dopaminergic neurones does not originate from striato-nigral fibres, as a kainic acid lesion in the striatum failed to affect nigral levels of AChE²¹. Therefore, afferent fibres from other regions may be involved. The situation is even more complex in the striatum, as in the rat, the dopaminergic terminals only contain about 12% of the entire striatal AChE⁴. Furthermore, kainic acid lesions in the striatum

showed that about 50% of the enzyme was distributed in intrinsic neurones²¹. Therefore, as initially suggested by Shute and Lewis²², various afferent nerve fibres could be rich in AChE. In fact, electrolytic lesions of the centro-median nucleus of the thalamus reduced AChE as well as choline acetyltransferase activities by 50% in the cat²³. Hence, AChE measured in superfusates of the substantiae nigrae and the caudate nuclei could originate from sites other than dopaminergic dendrites or terminals.

Previously, we demonstrated the existence of complex mechanisms involved in the reciprocal control of the activity of the two nigro-striatal dopaminergic pathways. Asymmetric changes in DA release were observed in both caudate nuclei after modifications of DA transmission in one substantia nigra²⁴, or after unilateral sensory stimulation²⁵. These were associated with opposite asymmetric changes in the dendritic release of DA in both substantiae nigrae (reminiscent of the changes in AChE release, Fig. 1). In contrast, symmetric responses in DA release were seen in the two caudate nuclei during modifications of GABAergic or glycinergic transmission in one substantia nigra¹⁹. The existence of two different polysynaptic pathways was postulated to account for the asymmetric and symmetric effects observed¹⁹. Surprisingly, in the same experimental condition, that is, the unilateral nigral application of potassium, two opposite patterns of response for AChE and DA release were seen in the four structures, one being asymmetric (AChE) and the other symmetric (DA). This indicates once more that various neuronal pathways are involved in the reciprocal regulation of the activity of neurones innervating the two substantiae nigrae and caudate nuclei.

Although the origin of AChE released from the substantia nigra and the caudate nucleus has still to be elucidated, these results demonstrate that AChE can be released in the CNS through processes involving nerve activity. It has been suggested that AChE could have a role other than that of inactivating ACh^{1,5}. Before this hypothesis can be verified, it must be established whether or not the distal changes in the release of AChE induced by the unilateral nigral application of potassium are associated with changes in cholinergic transmission. Finally, whatever the mechanisms are in the control of AChE release from both substantiae nigrae and caudate nuclei, they should be taken into account in our subsequent understanding of neuronal interactions within the basal ganglia.

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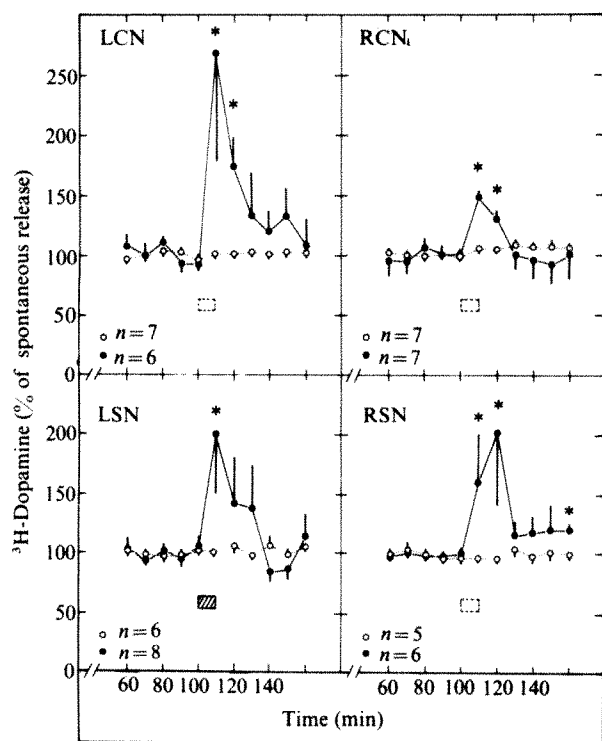


Fig. 3 Effect of the unilateral nigral application of potassium (30 mM) on the release of ³H-DA from the two substantiae nigrae and the two caudate nuclei. The experimental paradigm was essentially the same as described in Fig. 1, except that the artificial CSF delivered to the four push-pull cannulae contained L-[3, 5-³H]tyrosine (50 Ci mmol⁻¹, 50 μCi ml⁻¹, 1.2 ml h⁻¹) and that ³H-DA was estimated in 10-min successive superfusate fractions. Results were expressed as described in Fig. 1. The mean spontaneous release of ³H-DA in the left and right substantiae nigrae and left and right caudate nuclei being 0.65 ± 0.07, 0.57 ± 0.05 and 0.71 ± 0.08, 0.82 ± 0.10 nCi per 10 min, respectively. Data are the mean ± s.e.m. of results obtained with treated animals (●), *P* < 0.05 when compared with corresponding control values (○).

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Glycocalyx is not required for slow inward calcium current in isolated rat heart myocytes

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The importance of the slow inward calcium current (I_{si}) in the excitation-contraction coupling process of cardiac muscle is well documented^{1,2}. The current can be attributed mainly to a calcium translocation from the extracellular space into the cell or a subsarcolemmal compartment of it^{3,4}. I_{si} has been suggested to have its source in and to be controlled by the surface coat of the sarcolemma (glycocalyx)⁵. The glycocalyx is destroyed in myocytes dissociated from adult heart tissue with solutions containing low calcium, collagenase and hyaluronidase^{6,7} (Fig. 1). By comparing the I_{si} data obtained in isolated myocytes with those reported for trabeculae or papillary muscles, we have now obtained evidence suggesting that the glycocalyx is not important in the genesis of I_{si} .

Whereas low calcium solutions are necessary for the dissociation of the cells, analysis of I_{si} requires superfusion with a Tyrode solution containing 2–4 mM calcium. The cells were isolated using the method described in ref. 8. Ventricles were minced into 3-mm coarse fragments and incubated in a solution composed of 130 mM NaCl, 5.4 mM KCl, 1.2 mM $MgSO_4$, 11 mM glucose, buffered with 5 mM HEPES-NaOH to pH 7.4 and containing 1 mg ml⁻¹ collagenase (Sigma, Type C 2139) and 1 mg ml⁻¹ hyaluronidase. The cells were separated from the supernatant at 30-min intervals; the remaining tissue fragments were reincubated. The Tyrode solution contained 150 mM NaCl, 5.4 mM KCl, 3.6 mM $CaCl_2$, 1 mM $MgCl_2$ and 10 mM glucose, buffered with 5 mM HEPES-NaOH to pH 7.4. On return from low to physiological calcium concentrations rat heart muscle cells are susceptible to the 'calcium paradox' (ref. 9): the cells develop a strong contracture, become spherical and finally die. This problem was overcome by incubating the isolated cells in high K^+ , substrate-enriched solutions before stepwise elevation of calcium to a final concentration of 3.6 mM. Preincubation was at room temperature for 40 min in solution A (0.5 mM EGTA, 2 mM ATP, 2 mM succinate, 2 mM pyruvate, 2 mM β -hydroxybutyrate, 11 mM glucose, 20 mM taurine, 1.2 mM $MgSO_4$ and 100 mM K_2HPO_4 , adjusted with KH_2PO_4 to pH 7.4) and for 20 min in solution B (1 mM $CaCl_2$ = 0.3 mM free Ca^{2+} ; 2 mM ATP, 2 mM succinate, 2 mM pyruvate, 1.2 mM $MgSO_4$, 100 mM KCl and 30 mM K_2HPO_4 , adjusted with KH_2PO_4 to pH 7.4).

In about 50% of the rod-shaped cells we measured resting potentials between -75 and -85 mV, values similar to those reported previously^{10,11}. The cells did not beat spontaneously but they contracted in response to a stimulus. The action potentials (Fig. 2) resembled those of intact rat heart ventricular tissue. Voltage-clamp experiments were carried out with a single microelectrode technique (see Fig. 3a). The membrane current

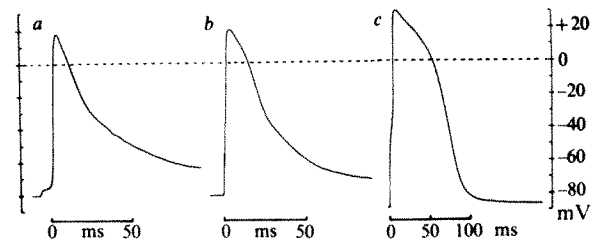


Fig. 2 Rat heart action potentials, intact tissue (a) compared with isolated myocytes (b, c). Isolated cells were viewed through an inverted microscope and impaled with 3 M KCl-filled microelectrodes. The configuration of the action potential differed appreciably: some cells displayed short-lasting action potentials (b) similar to those recorded from the intact trabecular (a), other cells displayed action potentials with a well pronounced plateau. Recordings from 20 cells revealed durations between 60 and 110 ms and amplitudes between 105 and 130 mV. All records were obtained in oxygenated Tyrode solution containing 3.6 mM $CaCl_2$. Temperature was 37 °C, stimulation frequency 1 Hz. Signals were digitised (10 bit per word) with a 2 K memory and stored on a digital tape recorder (ERDAC macrodyn).

evoked by a step depolarisation from -50 to 0 mV always started with a large initial outward surge and then declined within 10–12 ms to a minimum before reaching a steady outward level (Fig. 3b, see also Figs 4, 5). I_{si} was separated from the net current in the usual way^{3,4,12}. We subtracted the current waveform from the currents measured at the end of the clamp step and plotted this difference on a semi-log scale (Fig. 3d, ●). For clamp periods longer than 15 ms the plot shows a single exponential decay ($\tau_i = 10$ ms) which we associated with the inactivation of I_{si} . τ_i increased when the membrane was clamped to more positive potentials (Fig. 5c).

When inactivation of I_{si} was subtracted, the remaining currents changed during the first 15 ms of a clamp step with multiple time constants. An analysis of these currents (for time course of I_{si} activation) seemed impossible because of the limitations of the single electrode clamp technique (compare Fig. 3a).

An estimate of the voltage dependence of I_{si} amplitude was obtained by taking the difference in current between the quasi-steady state and the negative peak¹². The corresponding I - V curve (Fig. 4b, ●) reached a maximum at about 0 mV and crossed the V axis at a reversal potential of +50 mV ($+55 \pm 5$ mV; mean \pm s.e. from 5 cells). The compound D600 (ref. 13) greatly reduced I_{si} at all potentials (Fig. 4b, ×).

The voltage dependence of the steady-state activation (d_∞) and inactivation (f_∞) variables was analysed with the classical^{3,4} conditioning step program (Fig. 5a, b). The activation curve d_∞ started between -50 and -40 mV, increased e -fold per 22 mV (16 and 18 mV for two other cells) and reached saturation at 0 mV. Steady-state inactivation (f_∞) appeared between -40 and -30 mV, changed e -fold over 12 mV (11 and 12.5 mV for two other cells) and was complete around 0 mV. The curves intersect between -50 and 0 mV, suggesting a steady state I_{si} within this potential window.

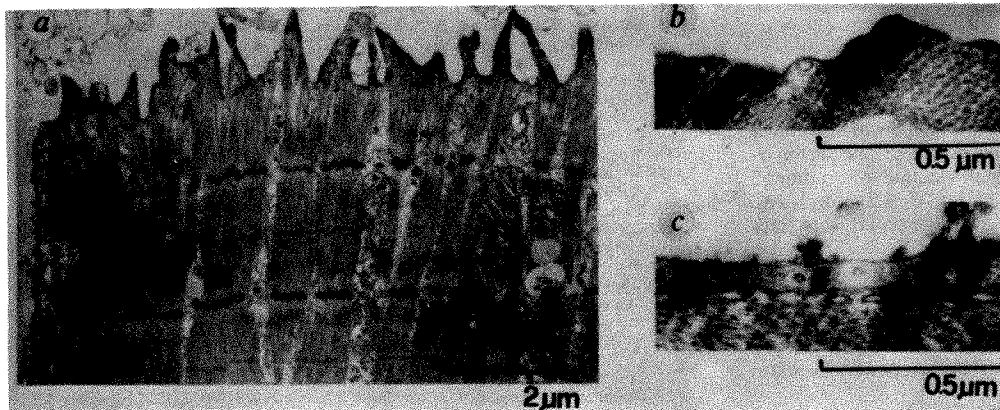


Fig. 1 Transmission electron micrographs of myocytes isolated from adult rat hearts and superfused with Tyrode solution. a Shows a longitudinal section of a myocyte separated at the intercalated disk. The normal arrangement of the myofilaments and the mitochondria are typical of unassociated heart muscle. The transverse section b shows a structural intact sarcolemma; no glycocalyx or other associated surface structures are present. Transverse section c was taken for comparison from non-dissociated rat heart tissue; here the sarcolemma is superimposed by a glycocalyx shown as a lanthanum-stained lamina about 70 nm thick. Specimens were handled by a convenient technique^{6,7}. For staining the surface coat¹⁷ (b, c) Tyrode solution containing 2 mM $LaCl_3$ superfused the preparations 30 min before fixation. In addition, four samples were stained with ruthenium red; again (as in b), no glycocalyx was present at the surface of the isolated myocytes (not illustrated).

Fig. 3 Voltage-clamp experiments carried out with a single intracellular microelectrode. An electronic current pump¹⁸ injected current and simultaneously measured the membrane potential, electronic compensation minimised the current-induced voltage drop at the electrode resistance which was reduced by bevelling to 2.5 M Ω . (It has been shown¹⁹ that currents 2 nA through bevelled microelectrodes produce no tip polarisation or rectification.) *a*, Evaluation of the method. A second (current-free) microelectrode was impaled 50 μ m from the site of current injection; it measured the potential V_1 . Whereas V_0 , the potential of the current-injecting electrode followed the command within 0.2 ms, V_1 shows a delay of about 2 ms. Afterwards, V_1 is stable and deviates from V_0 by -2.5 mV. *b*, Membrane currents in response to a depolarising clamp step. The holding potential of -50 mV inactivated the fast sodium current before the 120-ms step to 0 mV. The validity of this method is indicated by the finding that I_{Na} remained constant when tetrodotoxin was added (5×10^{-5} g l⁻¹) or Na⁺ was replaced by tetramethylammonium or Tris. *c*, After having depolarised the cell for different periods of time, repolarisation back to -50 mV evoked an inward-directed tail current with an amplitude which reflects the conductance change at 0 mV. The envelope of the tails follows the same time course as the net current recorded at 0 mV (see Fig. 4*a*). *b* and *c* were photographed from an oscilloscope in storage mode. *d*, A semi-log plot of the difference between the current waveform and the current level at the end of the depolarising clamp step. The current record shown in *b* is analysed with the filled circles, the envelope of the tail currents (*c*) with the open circles.

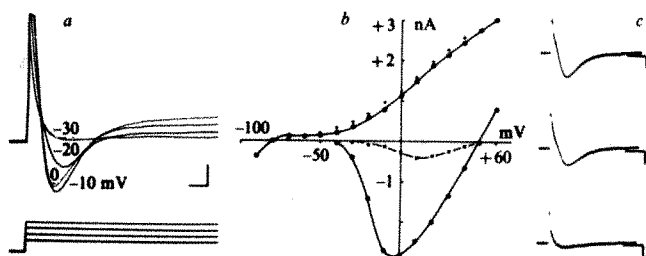
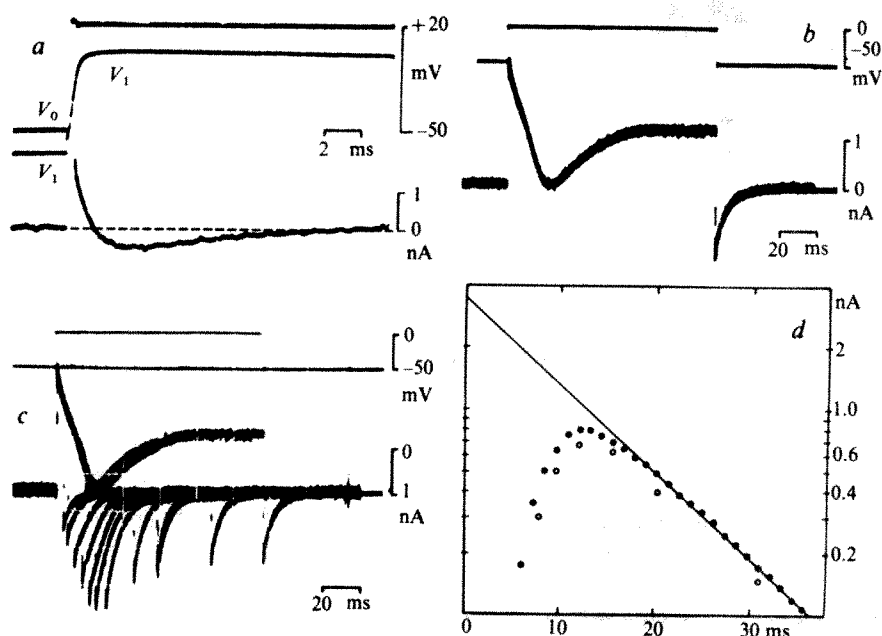


Fig. 4 (Above) Dependence of membrane currents on clamp potentials. The current tracings (*a*) were resolved into i_{Na} (outward current measured at the end of the 200-ms pulse which is not shown) and I_{Na} (peak negative current minus i_{Na}); values were plotted against the potential of the clamp step (circles in *b*). Exposure to 2 μ M D600 for 3 min (\circ , \bullet , before; $+$, \times , after application) strongly reduced I_{Na} (\times) but not i_{Na} ($+$). Currents evoked by a step to 0 mV before, 1 and 2 min after exposure to 2 μ M D600 are compared in *c*. Interference from the fast sodium current was minimised by imposing all voltage steps from a holding potential of -50 mV. Calibration marks in *a* and *c* indicate 10 ms and current from 0 to -1 nA.

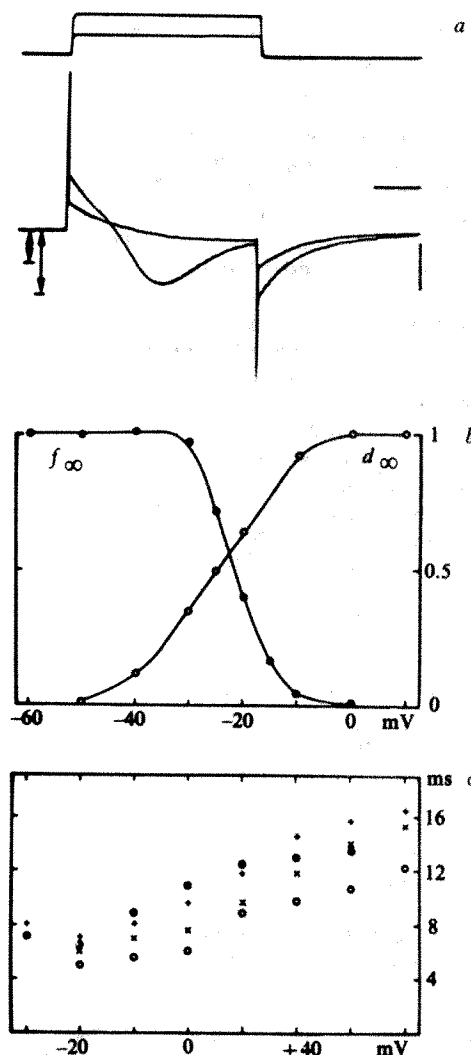


Fig. 5 (Right) Influence of the membrane potential on the conductance parameters d_{∞} , f_{∞} and τ . Activation was analysed at -50 mV; a short depolarising pulse (8 ms to -30 and -20 in *a*) activated the conductance to a degree which is proportional to the amplitude of the tail currents (arrows). Inactivation (not illustrated) was measured by imposing steps to +10 mV after 3-s pre-pulses to potentials between -70 and 0 mV. Having normalised the maximum effects to 1.0, the dimensionless variables d_{∞} and f_{∞} were plotted against the potential of the conditioning step (*b*). The inactivation time constant τ (*c*) was analysed according to Fig. 3*d*; the different symbols indicate four individual myocytes. Calibration marks indicate 4 ms and current from 0 to -1 nA.

Our data indicate that myocytes isolated from adult rat heart tissue can have electrical properties similar to those displayed by intact tissue. With regard to the slow inward calcium current, I_{si} , threshold, maximum amplitude and direction depended on membrane potential as in the intact tissue; the reversal potential is +55 mV, quite close to the values reported^{1,2}. Also, the influence of membrane potentials on the steady-state activation and inactivation curves is in good agreement with measurements on intact cardiac tissue from bovine or feline hearts^{1,2}. However, our τ_i values are similar to those estimated by us from currents recorded in rat heart ventricular trabeculae^{14,15}, that is, fast inactivation seems to be a peculiarity of the rat heart. This conclusion is supported by preliminary experiments with myocytes isolated from bovine heart tissue which revealed τ_i values of the order of 100 ms. We conclude that the glycocalyx, which is removed by the cell isolation procedure, is unlikely to be the structure originating or controlling the slow inward calcium current.

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Low intracellular pH and chemical agents slow inactivation gating in sodium channels of muscle

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Excitation of nerve or muscle requires an orderly opening and closing of molecular pores, the ionic channels, in the plasma membrane. During the action potential, Na channels are opened (activated) by the advancing wave of depolarisation, contributing a pulse of inward sodium current, and then are closed again (inactivated) by the continued depolarisation¹. As one approach both to obtaining molecular information on the Na channel and towards further defining the recently discovered kinetic interactions of the inactivation and activation gating steps^{2–4}, we have surveyed here the effects of chemical agents reported to slow or prevent Na channel inactivation. We find that many of the agents studied by others^{5–14} on invertebrate giant axons or vertebrate nerve act on our frog skeletal muscle preparation. In addition, we have discovered that simply lowering the intracellular pH nearly eliminates inactivation. The activation mechanism seems to resist modification.

Figures 1 and 2 show examples of a loss of inactivation gating following chemical treatments. The traces are families of sodium currents recorded from a cut piece of frog semitendinosus muscle fibre under voltage clamp by the Vaseline-gap technique¹⁵. The membrane was hyperpolarised for 56 ms and then depolarised to test potentials ranging from –90 to +70 mV in 16-mV intervals. In a normal muscle fibre bathed in Ringer's solution at pH 7 (Fig. 1a) or at pH 5 (Fig. 1e), the sodium currents have a transient time course, activating quickly and inactivating fully (at least 98%) during each 8-ms depolarising test pulse. However, after a 10-min exposure to 0.7 mM *N*-bromoacetamide (NBA) in the external medium (Fig. 1b), the inactivation process is modified, and sodium currents are still large at the end of 8 ms. Sodium channels close only after the membrane potential is stepped back to the holding potential, giving a brief inward sodium 'tail current'. Even with 75-ms test pulses, the currents in NBA-treated fibres fail to inactivate

completely (Fig. 2f) and the end of the pulse is followed by a prominent tail current (not faithfully recorded at the low sampling frequency used after the first 8 ms in the record shown). Similar modifications of inactivation gating are seen in a fibre treated for 36 min with internal iodate (Figs 1c, 2b) and in fibres treated for 38 min with 40 mM external formaldehyde (Fig. 2e) or for 43 min with 1.8 mM external glutaraldehyde (Fig. 2c) followed by another 6 min in 10 mM glutaraldehyde (Fig. 2d).

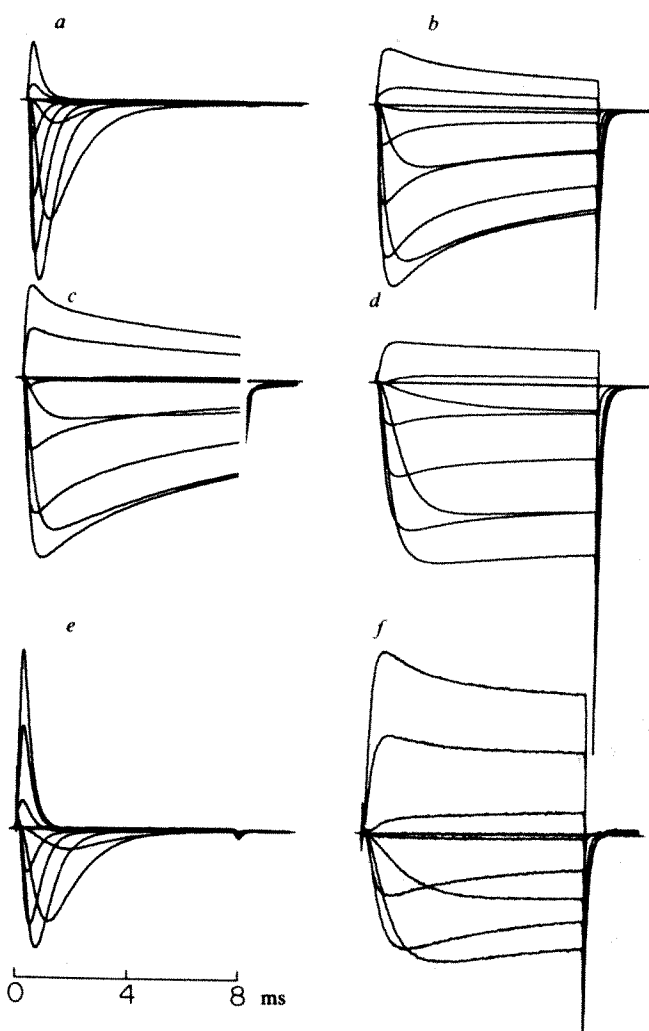
These observations with frog muscle parallel the reported effects on inactivation of 20–120 mM internal iodate in frog myelinated nerve^{5,6} and of 1 mM internal (but not external) NBA in squid giant axon⁷, and they are consistent with the action-potential-prolonging effect of 2 mM external glutaraldehyde in crayfish giant axons⁸. We also tested other agents reported to act on giant axons and obtained the results listed in the last two columns of Table 1. These agents seemed uniformly less promising for future use in our preparation, but most did slow the time course of inactivation.

In one series of experiments with fibres bathed in external solutions at pH 5, we found that inactivation remained normal when the buffer was 17 mM histidine (Fig. 1e) but became incomplete during 45 min when the pH 5 buffer was 28 mM acetate (Fig. 1f). This change could be slowly reversed, especially at an intermediate stage, by returning to low-pH histidine or to normal Ringer. On the hypothesis that acetic acid might cross the sarcolemma and act by acidifying the myoplasm, we tested the effect of lowering internal pH by placing 48–60 mM biphthalate buffer at one cut end of fibres bathed externally with flowing, well buffered solutions at neutral pH. Inactivation was nearly completely lost after 50 min with pH 4 buffer at one end and after 100 min with pH 5 buffer (Figs 1d, 2h). This treatment gave the most complete removal of inactivation of any we have tried and resulted in no loss of peak current. The effect could be further potentiated by external application of pH 5, acetate-buffered Ringer.

We also examined whether bisulphite ion, which has been reported to modify endplate channels¹⁶, also modifies gating in Na channels. When 25 mM bisulphite was applied for 100 min from inside or outside in a pH range of 6 to 9, there was no effect except a gradual reduction of current. However, if one cut end of the fibre was bathed for about 1 h in a pH 5.6 bisulphite solution and then the membrane was perfused externally with a pH 5.2 bisulphite Ringer's solution, inactivation was lost (Fig. 2g), and returned slowly after both bisulphite solutions had been washed away. We believe that external bisulphite acts here simply by accelerating the acidification of the myoplasm, perhaps by diffusion of the uncharged SO₂ species. Such synergism could be

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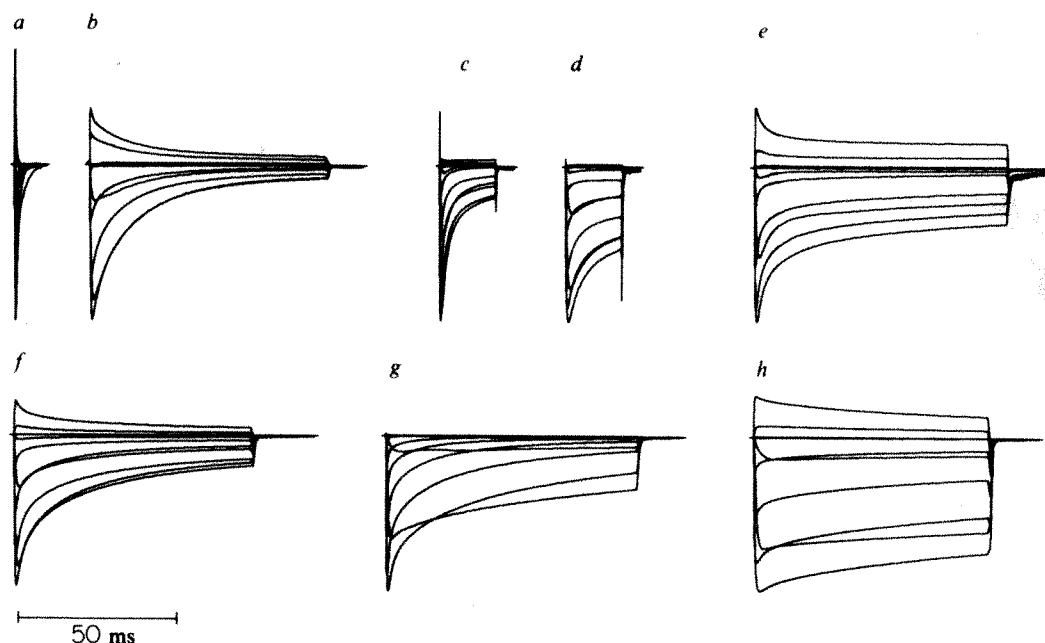
Fig. 1 Families of Na currents after treatments with agents that modify inactivation. Family *a* was recorded before, and family *b* at 8 min after, a 10-min exposure to 0.72 mM external NBA (in Ringer's buffered at pH 7 with 8 mM phosphate; internal solution: 60 mM KF, 60 mM CsF); fibre 6/19. Family *c* was obtained from fibre 6/20, at 14 min after the end of a 22-min internal application of iodate (60 mM NaIO₃, 60 mM CsF, then replaced by 20 mM Na phosphate buffer, pH 7, 100 mM CsF; external solution: Ringer's). The first 250 μ s of the tail current were not recorded. Records *d*, from fibre 6/6, were made at 103 min after the beginning of an internal treatment with pH 5, biphthalate buffer (48 mM K biphthalate, 24 mM NaOH, 60 mM CsF; outside: Ringer's). Family *e* was taken after 8 min in external Ringer's with 17 mM histidine buffer at pH 5; *f* was recorded later from the same fibre, 6/4/1, after a repeated, external application (total duration 42.5 min) of Ringer's with 48 mM acetate buffer at pH 5.1 (internal solution: 20 mM Na phosphate at pH 7, 100 mM CsF). Holding potentials -90 mV (*a-d*) or -74 mV (*e, f*); 8-ms depolarising pulses from -74 to +70 mV at intervals of 16 mV. Temperature 10-11 °C. Each record was scaled for the same amplitude of the largest inward (*a-d*) or outward (*e, f*) current; amplitudes were (*a-f*): 2.69, 0.93, 2.57, 4.64, 1.02, 0.272 μ A. The largest control currents for *c* and *d* were 3.86 and 5.55 μ A.



expected from all agents that can permeate to the myoplasm and there, by dissociation or other chemical reactions, generate free protons. In the test tube we found that added NBA, formaldehyde, glutaraldehyde or bisulphite lowers the pH of a histidine buffer solution within a few minutes from pH 7 to values lower than 4 by chemical reactions. We therefore cannot exclude the possibility that a slowly developing reduction of intracellular pH also contributes to the efficacy of these agents in muscle fibres.

Where do the chemical modifiers act? Two of the treatments we tried, iodate and low pH, were effective only from the inside of the membrane. External iodate up to 120 mM was totally without effect and low external pH gave only the known small effects¹⁷ seen in Fig. 1*e*. Other small, uncharged agents such as NBA and aldehydes were effective when applied outside, but as they can readily diffuse across membranes, they might also be acting inside. The chemical specificity of the agents we have tried has been discussed extensively elsewhere, and other

Fig. 2 Slowed Na inactivation seen with long step depolarisations. *a*, Records from a normal fibre, 6/20 (Ringer's outside; 60 mM NaF, 60 mM CsF inside). *b* Is the same fibre 16 min after the end of a 22-min internal exposure to iodate (same fibre as Fig. 1*c*). *c* Is taken from fibre 4/26 after 43 min external treatment with glutaraldehyde (1.8 mM in Ringer's; inside: 120 mM CsF), and *d* from the same fibre after a further 6 min exposure to 10 mM glutaraldehyde in Ringer's. Records *e* were taken from fibre 4/25 after 38 min external application of 40 mM formaldehyde (with 0.04% methanol) in Ringer's (inside: 120 mM CsF). Family *f* was recorded



17 min after the end of a 10-min external exposure to 0.72 mM NBA (same fibre as in Fig. 1*a, b*). *g* Is from fibre 4/20, which was treated for 72 min with internal bisulphite (19 mM NaHSO₃ in 120 mM CsF, pH 5.6), simultaneously exposed for 5 min (at 46-51 min) to external bisulphite (30 mM NaHSO₃) in Ringer's, pH 5.2, and then washed for 21 min with Ringer's. Records *h* were taken after 110 min of internal exposure to pH 5 biphthalate buffer (same fibre as in Fig. 1*d*). Same depolarisations as in Fig. 1. Temperature 10-11 °C. Currents were scaled to the same size; the maximum inward currents were (*a-h*): 3.85, 2.32, 0.51, 0.29, 0.63, 0.89, 1.99, 4.63 μ A; corresponding control currents were (*c-h*): 0.99, 0.99, 1.70, 2.69, 1.77, 4.64 μ A.

Table 1 Other experiments on frog muscle with agents reported to modify Na inactivation

Agent	Ref.	Modes of application	Our experiments	
			Depression of Na current*	Action on inactivation†
<i>N</i> -Bromosuccinimide (NBS)	7, 9	External, 1–2 mM	+	++
2, 4, 6-Trinitrobenzene sulphonic acid (TNBS)	10	External, 3–30 mM Internal, 60–120 mM (pH 7 or 9)	+	–
			++	+
4-Acetamido-4-isothiocyano-stilbene-2,2'-disulphonic acid (SITS)	10	External, 1–4.4 mM Internal, 11.5 mM	+	–
			++	+
Isethionylacetamide hydrochloride (IAH)	10	External, 3–10 mM Internal, 10–70 mM	–	–
			–	–
Glyoxal	11	External, 6–60 mM Internal, 11–60 mM	–	–
			–	+
Tannic acid	8	External, 2.2 mM Internal, 0.3 mM	++	–
			–	–
Picric acid (TNP)	13	External, 2.2 mM	++	+
High internal pH	14	Internal, pH 9.5–10	++	–

* Current depressed by less (+) or more (++) than one half.

† Inactivation slowed, but almost complete after 8 ms (+), or markedly incomplete after 8 ms (++).

authors^{7,10,11,14,18} have argued that modifications at tyrosine and arginine and reaction with lysyl- or N-terminal amino groups on the axoplasmic side of the membrane all remove Na inactivation.

Although inactivation gating was strongly modified by chemical treatments, activation gating seemed hardly affected. Figure 3 shows the voltage dependence of peak Na permeability and of the half-rise and half-fall times of the Na current during the test pulse. The normalised permeability-voltage curves are quite similar before (solid line) and after (dashed line) the chemical treatments except that with NBA, iodate and biphthalate (Fig. 3a, c, d) there is a small (<8 mV) shift to the left along the voltage axis. The half-rise times (triangles) are similarly

unchanged with NBA, formaldehyde and iodate (Fig. 3a–c), but are longer with the low internal pH (biphthalate, Fig. 3d). As is already clear, the fall times (circles) are greatly increased after all treatments, with low internal pH being the most potent.

These experiments confirm that inactivation and activation gating processes may be dissociated functionally, as if they are subserved by different parts of the channel. Perhaps the activation machinery is physically more deeply buried in the membrane and therefore less susceptible to chemical modification¹⁹. However, it must be remembered that all treatments except low internal pH also reduced the peak Na currents, and in some of these cases the reduction might reflect alterations in parts of the channel associated with activation.

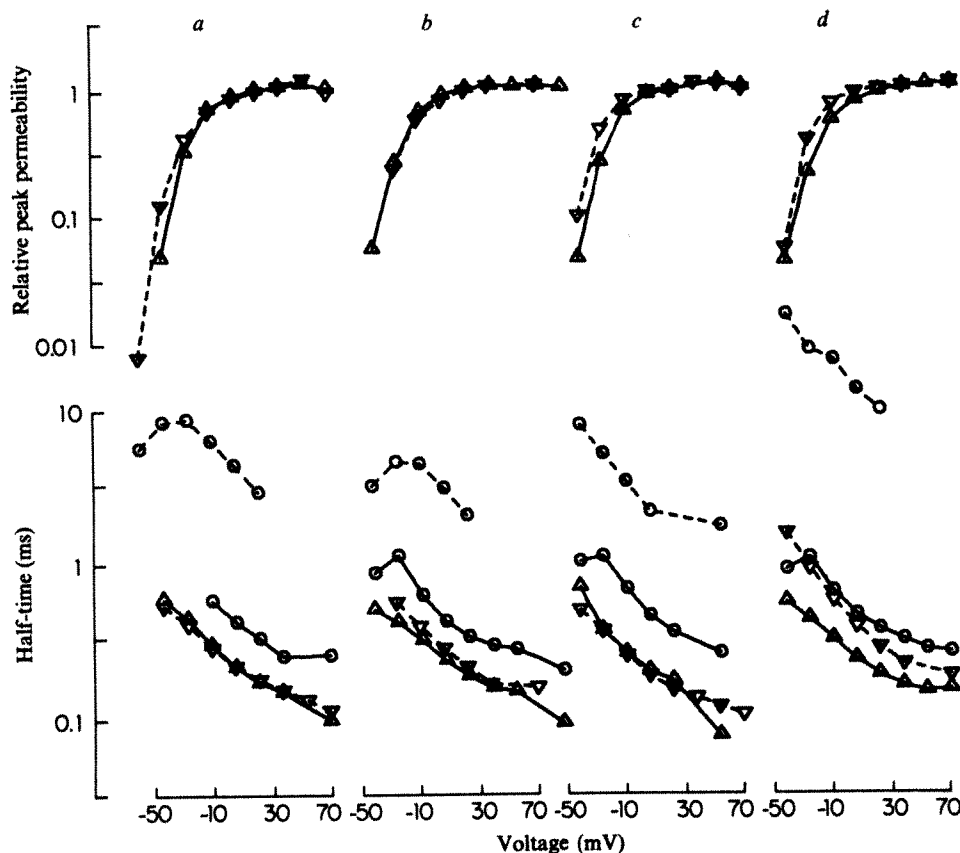


Fig. 3 Effects of agents on parameters of Na current. Upper: peak permeability, calculated from the Goldman flux equation²⁰; lower: half-times for rise and fall of Na current. Δ , Time to rise to half of peak value; \circ , time after peak to fall half-way to value at 75 ms. Controls are connected by solid lines and modified parameters by dashed lines; abscissa is the voltage of the test pulse. *a*, Before and after treatment with 0.72 mM external NBA (fibre 6/19, see Figs 1a, b, 2f). *b*, Before and during treatment with 40 mM external formaldehyde (fibre 4/25, see Fig. 2e). *c*, Before and after treatment with 60 mM internal iodate (fibre 6/20; Figs 1c, 2a, b). *d*, Before and during internal exposure to pH 5, biphthalate buffer (fibre 6/6; Figs 1d, 2h). Temperature 10–11 °C.

The chemical separability does not, however, exclude the possibility of interactions between the gating processes in normal channels: activation gates may still operate differently in inactivated and non-inactivated channels²⁻⁴.

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Distribution of membrane anionic sites on B16 melanoma variants with differing lung colonising potential

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The interaction of metastatic cells with their environment is mediated to a large extent by the cell surface¹⁻⁵. Although several biochemical differences between tumour cells with low or high metastatic potentials have been reported⁶⁻¹⁰, the specific surface characteristics associated with metastasis have not yet been identified. One distinctive feature of murine B16 melanoma variants with low (B16-F1, B16-F10^{Lr}) or high (B16-F10) lung colonisation potentials^{11,12} is their propensity to aggregate *in vitro* with other tumour cells (homotypic clumping)^{13,14}, or with host cells (heterotypic clumping)^{15,16}. The initial sites for membrane-membrane recognition, contact and subsequent interaction are thought to be associated with dense membrane anionic sites¹⁷⁻²⁰. In the experiments reported here we determined that the distribution of cell-surface dense anionic sites, examined ultrastructurally with the use of cationised ferritin (CF), is correlated with tumour cell aggregation *in vitro* and/or production of pulmonary tumour colonies following intravenous (i.v.) injection into syngeneic recipients.

The B16-F1 (parent, intermediate metastatic potential), B16-F10 (selected *in vivo*, high metastatic potential)¹¹ and B16-F10^{Lr} (selected *in vitro* for resistance to lysis by syngeneic lymphocytes, low metastatic potential)¹² were maintained *in vitro* as described previously¹². Cells from semiconfluent monolayer cultures were collected with a short (2 min) treatment of 2 mM EDTA in Ca²⁺- and Mg²⁺-free phosphate buffer saline (PBS), pH 7.2. The cells were washed in PBS and pipetted gently to dissociate any cell clumps. In all experiments, only cell suspensions of >90% viability, as measured by trypan blue exclusion, that were free of cell aggregates were used for *in vivo* or *in vitro* studies.

The biological behaviour *in vivo* of the cell lines was evaluated by i.v. injection of 5 × 10⁴ viable cells into groups of 10 mice

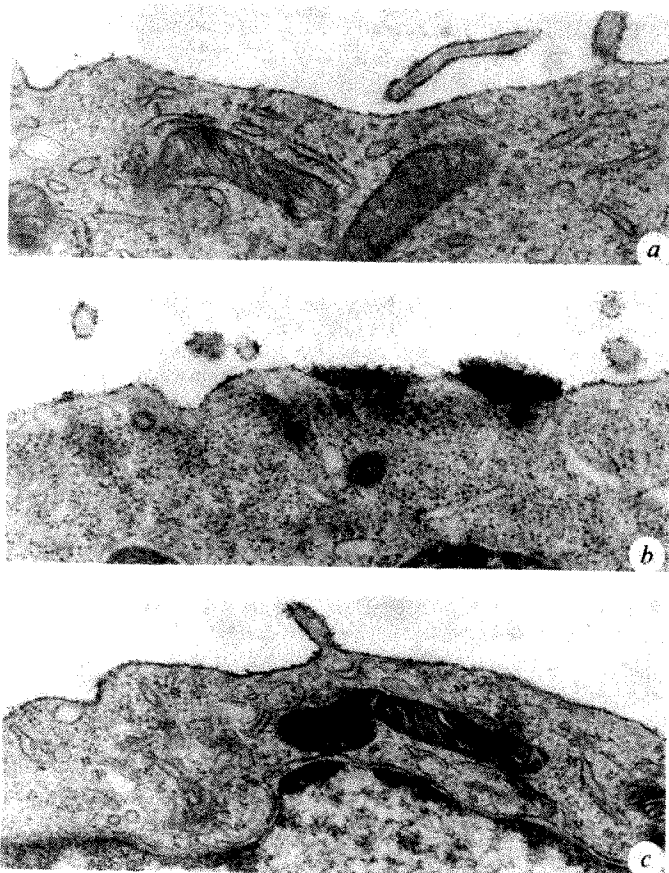


Fig. 1 Distribution of cationised ferritin on the cell surface of B16 melanoma cell lines: *a*, B16-F1; *b*, B16-F10; and *c*, B16-F10^{Lr}. Cell suspensions were fixed with 2.5% glutaraldehyde in Na-Veronal buffer (pH 7.4) for 60 min at 24 °C. Following washing three times with the same buffer the fixed cells were incubated for 3 min with 0.33 mg ml⁻¹ cationised ferritin (Miles Yeda)¹⁴. The unbound cationised ferritin was washed away and the cells were post-fixed with 1% buffered OsO₄ for 60 min at 24 °C and stained *en bloc* with a solution of 1% uranyl acetate. The cells were dehydrated with a graded series of ethanol followed by propylene oxide and embedded in Emix resin (Ted Pella, Inc.). Thin sections were obtained with an LKB ultramicrotome and analysed with the aid of Hitachi HU-12A electron microscope at an operating voltage of 75 kV. Serial sections of 600 Å through the CF clusters established that the observation was not due to an artefact of sectioning.

Table 1 Distribution of cationised ferritin clusters on surface of B16 melanoma cells

Cell lines	No. of cells with CF clusters/ no. of cells examined	Per cent of total
F1	38/180	21
F10	114/279	41
F10 ^{Lr}	51/180	28
F10*	27/122	22

Methods as described in Fig. 1. The results represent the average obtained from a random cell thin sections, and blind scoring. The experiments were repeated twice with cells grown from different (frozen) stock cultures with similar results.

* B16-F10 cell suspensions (1 × 10⁶ cells ml⁻¹) in 5% FCS in medium were placed in a 15-ml conical tube and rotated gently for 60 min at 37 °C. The aggregates were then allowed to sediment for 30 min at 24 °C. The upper third layer of the suspension was collected, washed with PBS, fixed and stained with CF.

Table 2 Homotypic aggregation of B16 melanoma cell lines

Cell line	Per cent cells in aggregates
F1	32
F10	52
F10 ^{Lr}	11
F10*	25

B16 melanoma cell suspension (1×10^6 cell ml⁻¹) in medium containing 5% FCS were placed into a 15-ml conical tube and rotated gently for 60 min at 37°C. An aliquot from each suspension was placed into a haemocytometer and the number of single cells per field was determined. The percentage of aggregates was calculated from the cell number in control experiments where the cells were incubated at 4°C.

* B16-F10 cell suspension was incubated for 30 min with 0.5 µm ml⁻¹ of neuraminidase, then washed and allowed to aggregate as above.

each. The mice were killed 21 days later and the number of tumour colonies in the lungs was determined. The median number of metastases produced by B16-F1, B16-F10 and B16-F10^{Lr} cells was 12, 130 and 2, respectively. These results agree with earlier reports^{6-8,11,12}. Quantitative determination of both intracellular and extracellular sialic acid²¹, demonstrated no differences among the three B16 lines as each line contained about 0.025 µmol of sialic acid per 10⁶ cells.

The distribution (topography) of the cell-surface anionic sites was examined by electron microscopy of cells treated with CF as described previously²². To avoid a possible artefact of clustering of the ligand on live cells²³, we examined the distribution of CF on glutaraldehyde prefixed cells. Striking differences in ferritin binding on the surface of the B16-F10 (high metastasis) and B16-F1 and B16-F10^{Lr} (low metastasis) were observed (Fig. 1, Table 1). Specifically, in over 70% of the B16-F1 and B16-F10^{Lr} cells the CF was bound to the cell surface as a continuous discrete layer where each CF particle was separated from an adjacent particle by ~100 Å. In contrast, in 41% of the B16-F10 cells the ferritin particles were clustered on the plasma membrane (Table 1). In only 21% of B16-F1 and 28% of B16-F10^{Lr} cells, were small clusters of CF particles found.

The frequency at which the cells in the population demonstrated dense cell-surface anionic sites correlates with the degree of *in vitro* homotypic aggregation (Table 2). In this system at least, homotypic aggregation *in vitro* is energy and fetal calf serum dependent, as at low temperatures (4°C) or in absence of serum no significant aggregation of B16 cells could be detected. Neuraminidase treatment (0.5 units ml⁻¹, 30 min at 37°C) of B16-F10 melanoma cells before the homotypic aggregation assay reduced the number of cells per aggregate (Table 2).

Not all cells in the B16-F10 suspension participate in the process of homotypic aggregation. The frequency of dense anionic sites among single (unaggregated) B16-F10 cells was

Table 3 Incidence of pulmonary metastases in C57BL/6 mice following the i.v. injection of B16-F10 melanoma cells

Source of B16-F10 cells	Median (range)* of pulmonary metastases
1. Control, total population†	63.5 (37-96)
2. Single cells recovered from aggregates‡	66 (14-146)
3. Single cells recovered from supernate§	29 (1-100), P < 0.001

* Ten mice per group were injected with 5×10^4 viable cells (>90%) in 0.2 ml of PBS. Pulmonary metastases were counted with a dissecting microscope 18 d after i.v. injection.

† B16-F10 cells were incubated in CMEM and 5% FCS for 60 min at 4°C and then washed with PBS.

‡ B16-F10 aggregates (Table 2) were sedimented and dissociated by gentle pipetting. The cells were washed in PBS.

§ Single cells obtained from upper third layer on the suspension (unaggregated cells).

|| Statistically significant group 3 versus 2 or 1.

remarkably similar (22%) to that observed in the B16-F1 and B16-F10^{Lr} suspensions. Evaluations of CF clusters on the surfaces of the cells within the aggregate could not be accomplished as the CF molecules did not penetrate the aggregates. In the final experiments we injected syngeneic mice i.v. with single cell suspensions consisting of B16-F10 cells (viability of >90%) from: the starting (unaggregated) population; those recovered from clumps; or those that did not participate in the aggregation process. The data of a representative experiment demonstrate that B16-F10 cells that did not participate in the homotypic aggregation process *in vitro* formed fewer pulmonary lung colonies than did single B16-F10 cells recovered from cell aggregates (Table 3).

In conclusion, we found no quantitative differences in total cellular sialic acid among three cell lines of the B16 melanoma with different metastatic propensities. In contrast, differences in the topography and display of dense anionic sites were detected among the lines. The visualisation of cell-surface dense anionic sites may be a useful tool for the investigation of subtle changes in the cell surface of metastatic cells. Such changes might account for the differences in the propensity of the B16 melanoma cells to form experimental pulmonary tumour colonies.

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Peanut lectin binding properties of germinal centres of mouse lymphoid tissue

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Peanut (*Arachis hypogaea*) lectin (PNL) has been shown to agglutinate the 90% of cells from murine thymus which are supposed to be immature cortical thymocytes¹. Further studies on the numbers of thymocytes binding fluorescein isothiocyanate conjugated PNL (FITC-PNL) confirmed the large proportion of PNL binding cells^{2,3}. In other organs such as bone marrow, spleen and peripheral lymph nodes, smaller proportions of PNL positive cells have been recorded^{2,3}. PNL-positive cells outside the thymus have been reported to be either Th1-positive or null cells^{2,3}. It has also been suggested that PNL

binding may be a marker for immaturity not only in relation to T lymphocytes² but also amongst haematopoietic stem cells⁴. Thus PNL binding as an aspect of lymphocyte differentiation is a matter of considerable interest. The current study describes the distribution of horseradish peroxidase-conjugated PNL (HRP-PNL) on frozen sections of mouse lymphoid organs. It seems that PNL binds to cells in germinal centres but not to those in some other areas containing activated lymphocytes. There is good correlation between the presence of PNL-binding germinal centres in frozen sections of lymphoid organs and the number of PNL-binding cells counted in cell suspensions from the same organs.

In 8–12-week-old male CBA mice the proportions of lymphocytes which were able to bind FITC-PNL in cell suspensions from thymus, spleen, peripheral lymph nodes and bone marrow (Table 1) were found to be similar to those previously reported^{2,3}. However, the observation that 20% or more of Peyer's patch lymphocytes bind PNL led to the investigation of the localisation of the PNL-binding cells in Peyer's patches using frozen sections stained with HRP-PNL (Fig. 1a). It can be seen that there is a close correlation between the PNL-binding area and the germinal centre which can be observed in sections conventionally stained with haematoxylin and eosin (H&E) (Fig. 1b). Comparable results were obtained using FITC-PNL or H&E on frozen sections of rat Peyer's patches. Peripheral lymph nodes which in unstimulated laboratory mice usually lack germinal centres, did not stain with HRP-PNL but the few germinal centres to be found in spleen from unstimulated mice did prove to be PNL-positive. So far all germinal centres have bound PNL but it should be stressed that much more material must be examined before any great weight can be put on this finding. PNL binds to terminal galactosyl residues with the highest affinity for the sequence β -D-galactose-(1 \rightarrow 3)D-N-acetylgalactosamine⁹. The binding of PNL to germinal centres was completely abrogated in the presence of 0.1M galactose but not 0.1M glucose or mannose. Thus the binding of PNL to germinal centre cells is probably to terminal galactose residues on the cell surface.

Germinal centres contain dividing cells and their lymphocytes are pyronin-positive, indicating a higher degree of metabolic activity than is present in resting lymphocytes¹⁰. It was therefore of interest to determine whether PNL would bind to other activated lymphocyte populations. Local stimulation with the

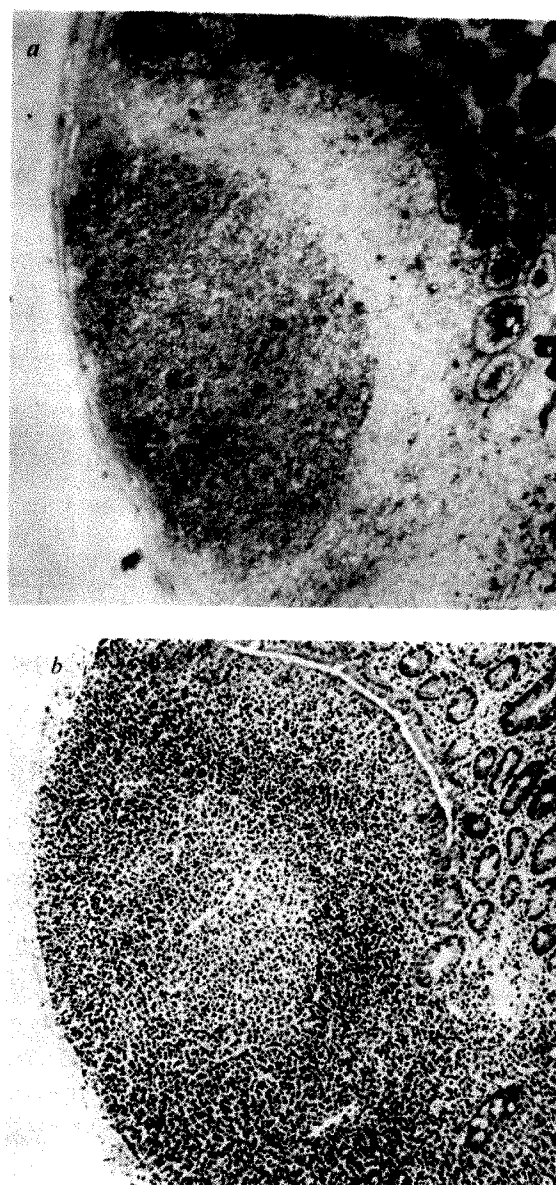


Fig. 1 Binding of HRP-PNL on frozen sections of murine Peyer's patches (a) and section from the same block conventionally stained with H & E (b). Peanut lectin was prepared according to Table 1 legend. HRP-PNL conjugate was prepared according to the method of Nakane⁸. Frozen sections (4- μ m thick) of mouse Peyer's patch were air dried, and stained with HRP-PNL (20 μ g PNL per ml) for 30 min at room temperature. Sections were washed three times in PBS and staining was developed using 3, 4, 3', 4'-tetra-aminobiphenyl hydrochloride. Sections were washed again in PBS, dehydrated and mounted. Magnification $\times 105$.

Table 1 Percentage of FITC-PNL binding cells in lymphoid organs of unstimulated CBA mice

Expt no.	1	2	3
Peripheral lymph node	4.0	3.0	
Spleen	6.0	5.0	
Thymus	76.0	82.7	88.0
Bone marrow	4.0		
Peyer's patch	26.7	26.0	19.4

Peanut lectin was prepared according to the method of Lotan *et al.*⁵, with the major exception that the final affinity purification step was performed using a column of lactose bound to epoxy activated Sepharose 6B from which the lectin was displaced by 50 mM galactose. The preparation possessed haemagglutinating activity of 31,000 units per mg towards neuraminidase-treated human erythrocytes, and showed only one protein band on disc electrophoresis at pH 8.6. PNL was conjugated to FITC⁶ in the presence of galactose to produce a FITC/PNL molar ratio of 2.7. Cell suspensions were prepared as previously described⁷ and 100 μ l of cells at 10^7 per ml in PBS containing 0.05% sodium azide were mixed with 100 μ l FITC-PNL (50 μ g PNL per ml) in phosphate-buffered saline (PBS) containing azide. After 30 min incubation at 37°, the cells were washed three times and resuspended in 100 μ l of PBS containing azide. Cells were examined under fluorescence optics using a Zeiss epifluorescence condenser IV/F on standard 18 microscope. Only viable cells (distinguishable from dead cells under phase contrast) with strong membrane fluorescence were scored as positive. In each experiment (done on different days) the relevant organs from at least four mice were pooled, tubes were counted in duplicate and the mean is shown. 200 cells per tube were scored.

contact sensitising agent, oxazolone, results in intense T-cell proliferation in the paracortex of the draining lymph nodes at 3 days¹¹. Frozen sections of such nodes did not bind PNL to an enhanced degree nor was it possible to detect an increase in the numbers of PNL-binding cells in cell suspensions from lymph nodes activated in such a manner (Table 2). However, at later times (days 7–15) increased numbers of PNL-binding cells were observed (Table 2). This increase was correlated with the time of appearance of germinal centres. No increase in the numbers of PNL-binding cells was found in spleen cells which had been activated *in vitro* with either lipopolysaccharide or with phytohaemagglutinin (Table 3). Thus PNL does not bind to activated lymphocytes *per se* but only to cells in particular anatomical locations. The fact that PNL-binding cells can be recovered from unfixed cell suspensions and demonstrated by agglutination¹ suggests that PNL is membrane binding.

Table 2 Percentage of FITC-PNL binding cells in the axillary lymph nodes of mice stimulated with oxazolone

	Days after oxazolone	No. of mice	Mean
Expt 1	0	3	4.0 ± 0.6
	3	4	3.4 ± 1.5
	7	2	12.3 ± 1.0
	13	1	13.9
	17	1	7.4
Expt 2	0	4	2.8 ± 1.2
	4	4	3.3 ± 0.9
	11	4	15.3 ± 3.4
	15	4	19.2 ± 6.0

PNL and FITC-PNL were prepared as described in the legend to Table 1. Mice were painted on the shaved flanks with 3 mg of 4-ethoxymethylene-2-phenyl-oxazolone (oxazolone, BDH) dissolved in 100 µl of ethanol. At various times after immunisation, mice were killed and cell suspensions from the draining axillary lymph nodes were prepared, they were washed twice and resuspended at a concentration of 10^7 per ml in PBS containing 0.05% sodium azide. The number of FITC-PNL binding cells was estimated as described in Table 1. Results are the mean ± s.d. from individual mice.

Table 3 Percentage of FITC-PNL binding cells in spleen cultured with LPS or PHA

	24	48	72
LPS	4.9 ± 1.6	4.2 ± 1.3	2.8 ± 1.0
PHA	4.9 ± 0.8	5.3 ± 3.4	4.8 ± 3.0

Spleen cells were cultured in RPMI1640 containing 10% fetal calf serum, 300 mM glutamine, 200 unit ml⁻¹ penicillin and 150 µg ml⁻¹ streptomycin, in 1 ml cultures at a concentration of 2×10^6 cells per ml with phytohaemagglutinin (PHA, Wellcome, 2.5 µg ml⁻¹) or 4×10^6 cells per ml with lipopolysaccharide (LPS) from *Escherichia coli* 055:B5 (Difco, 50 µg ml⁻¹). After culturing (at 37 °C in an atmosphere of 7% CO₂, 10% O₂, 83% N₂) for 24, 48 or 72 h, cells were collected and the numbers of FITC-PNL binding cells were estimated as described in Table 1. Results are the mean ± s.d. from four replicate cultures. Control cell suspensions of uncultured spleen revealed 6.0 ± 2.0, 5.5 ± 1.7 and 4.7 ± 1.1% FITC-PNL binding cells at 24, 48 and 72 h respectively. A control thymocyte suspension done at 72 h revealed 82% PNL binding cells.

The origin of cells in germinal centres has not been fully determined. Weissman¹² has shown that there are some T cells present by the use of fluorescinated anti-T cell antiserum. However, neither the numbers nor the pattern of dispersion of these cells corresponds to that seen with PNL. Thus the PNL-positive cells are unlikely to be mainly T lymphocytes. In the same study Weissman demonstrated that a substantial proportion of the area of germinal centres was immunoglobulin positive. This finding must be viewed with some caution before it is assumed that many or all of the cells in germinal centres are surface immunoglobulin-positive B lymphocytes. It is known that, in at least some germinal centres, there are dendritic reticular cells which bind antigen-antibody complexes and which have cell membranes with vast processes on which the complexes are to be found¹³. Preliminary studies in this laboratory with double staining techniques on cell suspensions from Peyer's patches have shown that some of the PNL-positive cells appear to be surface immunoglobulin positive, whereas others are not obviously so. Thus the anatomic origins of PNL-positive germinal centre cells are not clear, although it seems likely that some or all of them are B lymphocytes.

It is an intriguing coincidence that cortical thymocytes and germinal centre cells bind PNL. These observations, together with Reisner's suggestion⁴ that PNL binding is a marker of immaturity among T cells and cells of the haematopoietic stem cell population, again raise the possibility that germinal centres harbour a population of immature B cells^{14,15}. Use of PNL to separate germinal centre cells should allow their properties and functions to be characterised further.

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Protective monoclonal antibodies recognising stage-specific merozoite antigens of a rodent malaria parasite

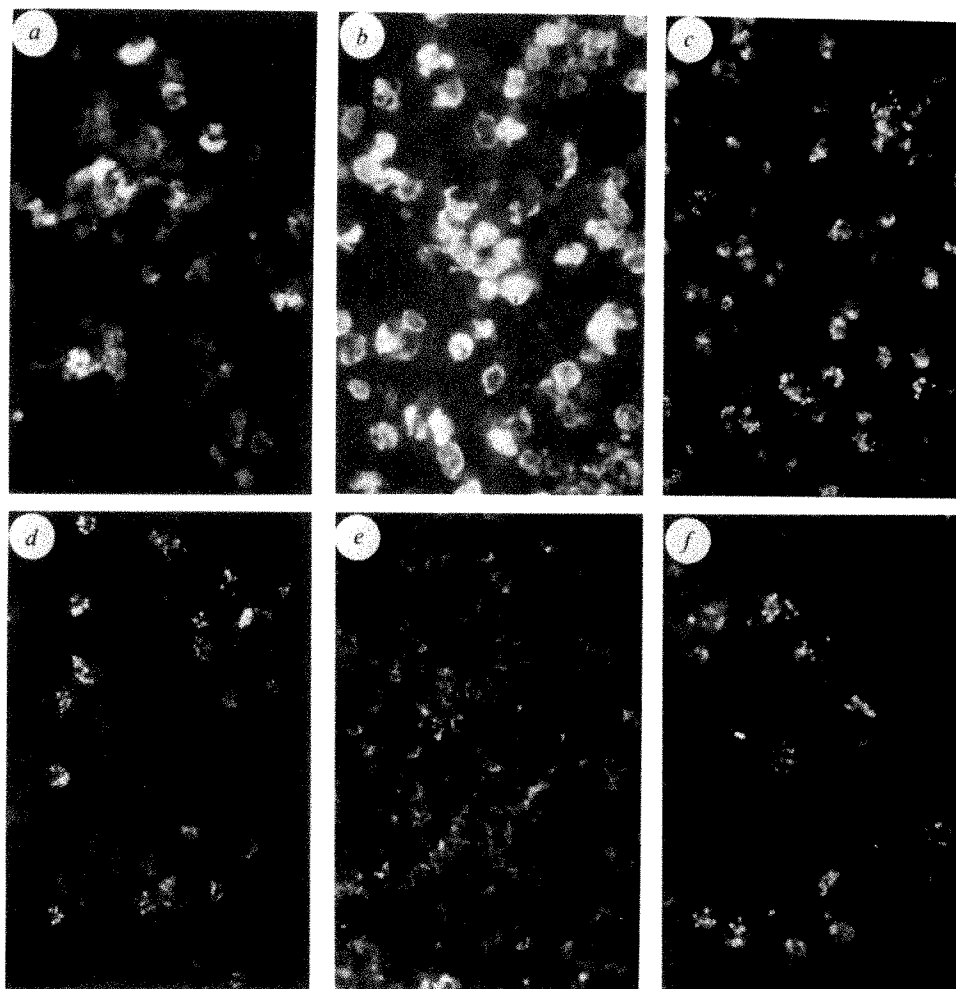
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Immunity to malaria is mediated, at least in part, by antibody. Resistance to infection has been passively transferred with immune serum or its immunoglobulin fraction in human¹, simian² and rodent^{3,4} malaria. However, because of the structural and antigenic complexity of the malaria parasites^{5,6}, it has proved difficult to identify and characterise those parasite antigens against which protective antibody is directed. We have produced several hybrid cell lines secreting monoclonal antibodies against the rodent malaria parasite, *Plasmodium yoelii*, and we now report that, of the antibodies tested, only those specific for antigens exclusive to the merozoite were protective in passive transfer experiments. Other anti-*P. yoelii* monoclonal antibodies, apparently recognising antigens in the membrane of the infected erythrocyte, were not protective on passive transfer. The protective monoclonal antibodies should be useful in the isolation of the important antigens of this parasite.

Spleen cells from two *P. yoelii*-immune BALB/c mice were fused with P3-NS1/1-Ag4-1 myeloma cells in the presence of polyethylene glycol⁷. The cells were dispensed into 144 tissue culture wells in 2-ml volumes of hypoxanthine-aminopterin-thymidine-selective medium⁸, using RPMI-1640 medium supplemented with 10% fetal calf serum as a base⁹. After 10 days, the culture supernatants were tested for *P. yoelii*-specific antibody by indirect immunofluorescence (IIF). Of 143 cultures tested, 38 were positive, 20 producing antibody apparently directed against the infected erythrocyte membrane and 18 producing antibody against the parasite itself. From the initial screening, five distinct IIF specificities were detected. Cultures representative of each of the IIF specificities were expanded in selective medium without aminopterin, and hybridoma lines were subsequently cloned in semi-solid agar overlaid with medium.

Fig. 1 IIF staining reactions produced by: *a*, whole anti-*P. yoelii* immune serum diluted 1 in 1,000, and *b-f*, 1 in 10,000 dilutions of sera from mice carrying ascites tumours derived from cloned hybridoma cell lines: *b*, WIC 25.1; *c*, WIC 25.23; *d*, WIC 25.37; *e*, WIC 25.54; *f*, WIC 25.77. Supernatants from cultures of these lines produced IIF reactions identical to those produced by the sera. To produce monoclonal antibodies, 2×10^8 spleen cells from BALB/c mice recovered from mild *P. yoelii* 17X infection and rechallenged three times with virulent *P. yoelii* (YM strain) were mixed for fusion with 2×10^7 P3-NS1/1-Ag4-1 myeloma cells⁹. To prepare antigen for the IIF test, BALB/c mice were infected with virulent *P. yoelii* 24 h before subcutaneous injection with cyclophosphamide (200 mg per kg). After 4 days, when parasitaemia was >60%, infected blood was enriched for schizonts by centrifugation through isopaque/Ficoll¹² (2,000g for 20 min at 20 °C). The enriched upper layer of cells was washed in phosphate-buffered saline (PBS), diluted 1 in 100 in PBS, and applied dropwise to glass slides. The antigen preparation was allowed to dry at room temperature, then slides were stored at -20 °C. Giemsa staining of preparations showed that all red cells were parasitised (30% schizonts, 70% trophozoites approximately). Free merozoites were also observed. The procedure for the IIF test has been described elsewhere¹³; in the present study, background fluorescence was minimised by using immunoabsorbant-purified rabbit anti-mouse Ig. ($\times 180$).



The five cloned lines secreted antibodies covering the range of IIF specificities originally detected. Whereas polyspecific serum from immune mice produced a generalised staining of *P. yoelii*-infected erythrocytes in the IIF assay, the monoclonal antibody specificities produced characteristic, restricted patterns of fluorescence (Fig. 1). Thus, with specificity 25.1, the membranes of a subpopulation of infected erythrocytes fluoresced, whereas specificity 25.54 bound to the membranes of all infected erythrocytes with a distinct patchy staining pattern. Specificity 25.23 bound to an antigen common to all developmental stages of the blood form of the parasite. Specificities 25.37 and 25.77 bound to antigens only present in merozoites, free and within mature schizonts. All merozoites in the antigen preparation apparently reacted with the two merozoite-specific antibodies, but the

Table 1 Characteristics of the anti-*P. yoelii* monoclonal antibodies secreted by five cloned hybridoma lines

Hybridoma line	IIF specificity of secreted antibody	IIF cross-reactivity against <i>P.v. petteri</i>	Immunoglobulin subclass
WIC 25.1	Infected erythrocytes	+	IgG2a
WIC 25.23	Trophozoites, schizonts and merozoites	+	IgG2a
WIC 25.37	Merozoites	-	IgG1
WIC 25.54	Infected erythrocytes	+	IgG3
WIC 25.77	Merozoites	+	IgG2a

IIF specificity was determined by testing against *P. yoelii*, YM strain. For immunoglobulin subclass determination, concentrated culture supernatant samples were tested by double diffusion in agar against rabbit antisera specific for the mouse immunoglobulin subclasses IgM, IgG1, IgG2a, IgG2b, IgG3 and IgA.

possibility that a minor subpopulation of merozoites failed to react could not be excluded.

The antibody products of the five lines were characterised with respect to immunoglobulin subclass by double diffusion using subclass-specific antisera. All were found to be of the IgG class (Table 1). They were also screened for cross-reaction against uninfected BALB/c erythrocytes and against a heterologous rodent malaria parasite, *Plasmodium vinckei petteri*, by IIF. None reacted with the uninfected erythrocytes but all except the merozoite-specific antibody secreted by line WIC 25.37 cross-reacted with *P.v. petteri* antigens (Table 1).

Serum pools containing high titres of anti-*P. yoelii* monoclonal antibodies were obtained from hybridoma tumour-bearing BALB/c mice (Table 2). These sera were tested in passive transfer experiments for their effect on the course of virulent *P. yoelii* infection (Fig. 2). Mice receiving monoclonal antibody specificities 25.1 and 25.54, apparently directed against infected erythrocytes, were not protected against *P. yoelii*. Neither was protection transferred with serum containing a high titre of specificity 25.23, directed against an antigen common to all developmental stages of the asexual erythrocytic parasite (trophozoites, schizonts and merozoites). However, transfer of sera containing the antibodies specific for late-appearing merozoite antigens, specificities 25.37 and 25.77, resulted in inhibition of *P. yoelii* parasitaemia, and none of these mice died. Whole immune serum was even more effective, causing the elimination of patent parasitaemia within 48 h of transfer. The protective activity of the monoclonal antibodies was not restricted to one IgG subclass, as specificity 25.37 was an IgG1 and specificity 25.77 was an IgG2a.

The eventual recovery of those groups treated with monoclonal anti-merozoite antibody was probably not mediated by the transferred serum, but rather by the recipients' immune

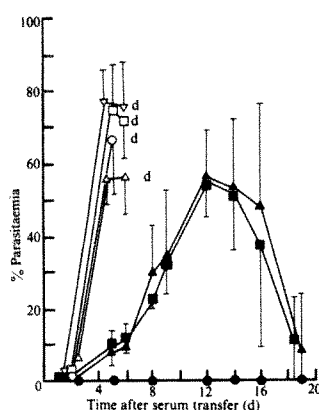


Fig. 2 The course of *P. yoelii* infection in groups of five BALB/c mice injected intraperitoneally with 0.5 ml of normal mouse serum (○), *P. yoelii*-hyperimmune serum (●), or serum from mice carrying tumours derived from cloned hybridoma lines: WIC 25.1 (△), WIC 25.23 (□), WIC 25.37 (▲), WIC 25.54 (▽) and WIC 25.77 (■). Serum was transferred when parasitaemia had reached 0.01–0.1%. Mean survival times are indicated (d). The vertical bars indicate standard deviations.

response. The direct effect of the transferred antibody was seen during the week following transfer, when the normally fulminating *P. yoelii* YM infection was retarded, following a course more typical of self-limiting *P. yoelii* 17X infection.

Inhibition of red cell invasion by merozoites may involve the blocking by antibody of receptor determinants on the merozoite surface. A polyspecific antiserum would be expected to be more efficient in receptor blocking than a monospecific antiserum. This may explain the greater degree of protection transferred with whole hyperimmune serum than with serum containing a single anti-merozoite specificity. An interesting alternative possibility is that the delayed parasitaemia observed in mice treated with specificities 25.37 and 25.77 was due to the emergence of a population of merozoites not reacting with these antibodies.

It is not known whether monoclonal specificities 25.37 and 25.77 recognise distinct merozoite antigens or different determinants on the same antigen. They give similar IIF staining reactions against *P. yoelii* (Fig. 1), but the observation that specificity 25.77 is cross-reactive with *P. v. petteri* indicates that this antibody possesses a binding specificity distinct from that of the other protective monoclonal antibody species. Using monoclonal antibodies and immunoprecipitation, we are now in the process of identifying the parasite antigens involved.

Our findings do not support the view that antibodies specific or parasite antigens present in the membrane of the infected erythrocyte are protective¹⁰, although they do indicate the presence of at least one erythrocyte membrane-localised *P. yoelii* antigen, against which a substantial but apparently non-protective antibody response is made in the immune host. However, we have tested only two membrane-specific monoclonal antibodies, and our results do not rule out the possibility

that other membrane-specific antibodies may prove protective. Furthermore, although specificities 25.1 and 25.54 bound to the membranes of infected erythrocytes in fixed smears, they did not bind to infected erythrocytes in suspension, and so the antigens recognised by these antibodies are probably not externally expressed on infected erythrocytes. Nevertheless, protection was transferred with the anti-merozoite monoclonal antibodies, and it therefore seems that in the case of *P. yoelii*, as with *Plasmodium knowlesi*¹¹, antibody-mediated protection against malaria acts at the level of the free merozoite, inhibiting invasion of host erythrocytes.

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Copper metallothionein, a copper-binding protein from *Neurospora crassa*

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Copper is an essential constituent of many proteins which participate in biologically important reactions¹. In contrast to iron, where different metal storage and transport proteins have been extensively characterised, the existence of copper proteins serving such functions is still a matter of controversy^{2–4}. Studies on the biosynthesis of tyrosinase from *Neurospora crassa* with respect to the copper status of this fungus have shown that this organism accumulates copper with the concomitant synthesis of a small molecular weight copper-binding protein. This protein is now shown to have a striking sequence homology to the zinc- and cadmium-containing metallothioneins from vertebrates⁵. Growth experiments suggest that this molecule fulfills several important physiological functions in this organism such as copper storage, copper detoxification and provision of copper for tyrosinase.

When *N. crassa* is grown in the presence of 0.5 mM of CuSO₄ in the culture medium most of the intracellular copper is found in a high molecular weight fraction during the logarithmic growth period (Fig. 1). However, in the stationary phase about 10% of the copper taken up by the cells accumulates in the form of a low molecular weight protein (Fig. 1). This copper-binding protein was isolated from lyophilised mycelium (obtained as described in Fig. 1 legend) by gel filtration of the crude extract on Sephadex G-50 and subsequent chromatography on DEAE-cellulose. The pure protein is composed of only seven different amino acids with a strikingly high content of cysteine (28%), serine (28%) and glycine (24%). The amino acid composition is: Asx, 2.9; Ser, 6.9; Gly, 6.0; Ala, 1.0; Cys, 6.9 (determined as carboxymethylcysteine); Lys, 1.0. The protein binds a total of 6 g atoms of copper per mw of 2,200. The copper-free apoprotein can be obtained by exposure of the holoprotein to low pH (<pH 1.0) and subsequent metal removal by gel filtration. The homogeneity of the molecule was further corroborated by the

Table 2 IIF titres of sera used in passive transfer experiments

Serum source	Anti- <i>P. yoelii</i> IIF titre
BALB/c mice carrying hybridoma WIC 25.1	1:30,000
BALB/c mice carrying hybridoma WIC 25.23	1:32,000
BALB/c mice carrying hybridoma WIC 25.37	1:128,000
BALB/c mice carrying hybridoma WIC 25.54	1:30,000
BALB/c mice carrying hybridoma WIC 25.77	1:128,000
Immune BALB/c mice*	1:70,000
Normal BALB/c mice	<1:20

Anti-*P. yoelii* IIF titre is expressed as the highest dilution giving positive fluorescence.

* Donors had recovered from mild *P. yoelii* 17X infection, and serum was taken 7 days after challenge with virulent *P. yoelii* (YM strain).

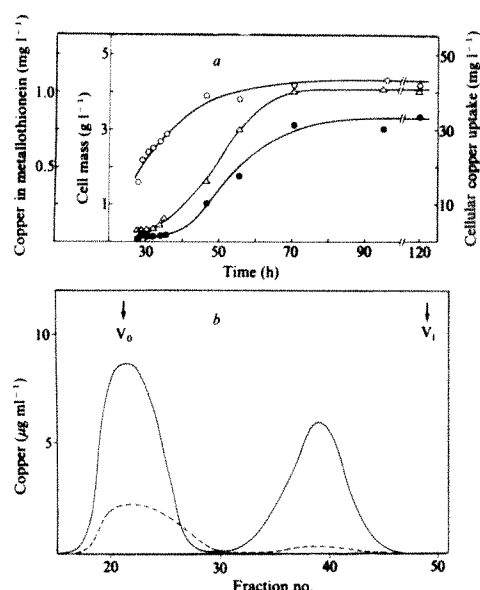


Fig. 1 *a*, Growth curve of *N. crassa* in the presence of 0.5 mM CuSO_4 in the culture medium. The amount of copper metallothionein (●), the uptake of copper from the medium (Δ) and the increase of cell mass as dry weight (○) are shown as a function of time. Cultures of 400 ml half-strength Vogel medium N (ref. 17) supplemented with 0.5 mM CuSO_4 were grown in 1-l Erlenmeyer flasks at 25 °C by shaking at 105 oscillations min^{-1} on a reciprocal shaker. Cells were collected by filtration through cheesecloth, washed with distilled water and the lyophilised mycelium extracted with 50 mM phosphate, pH 7.2. The concentration of copper metallothionein was determined by gel filtration of crude extracts on Sephadex G-50 in 10 mM Tris-HCl, pH 8.0, and subsequent measurement of the metal content of the fraction eluting at an apparent mw of about 3,000. Copper analyses were carried out by atomic absorption spectroscopy. All parameters are expressed per l of culture medium. *b*, Sephadex G-50 elution profiles of crude extracts from 35-h (---) and 70-h (—) old mycelium. Experimental details as in *a*.

determination of its primary structure using automated Edman degradation of the pyridine-ethylated apoprotein¹⁰. Partial sequence analyses of the tryptic peptides derived from the S-aminoethylated apoprotein¹¹ and amino acid analyses of tryptic peptides were in agreement with the results from the automated Edman degradations. The amino acid sequence is strikingly similar to those of the amino-terminal region of the Cd/Zn metallothioneins from man, horse and mouse¹²⁻¹⁴ (Fig. 2). The cysteinyl residues of the *Neurospora* copper-binding protein occupy exactly the same positions as the first seven cysteinyl residues in the amino-terminal sequence region of the Zn/Cd metallothioneins. In addition, serine residue 12 is invariant in all four proteins. Note the complete lack of aromatic amino acids in the copper-binding protein from *N. crassa* as well as in all the Cd/Zn metallothioneins sequenced so far⁹. Because of these common structural features we categorise the *Neurospora* copper-binding protein as a member of the superfamily of metallothioneins⁹. Further, it is suggested that the gene of the *Neurospora* copper-binding protein is related to the primordial gene of the vertebrate metallothioneins which might have evolved to the well characterised present day forms through gene duplication.

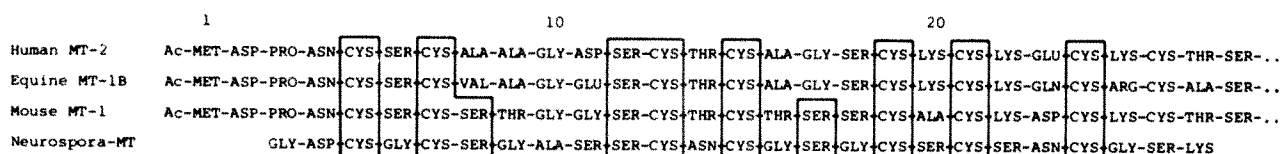


Fig. 2 Comparison of sequences of *Neurospora* copper metallothionein and Zn/Cd metallothioneins from human¹², equine¹³ and mouse¹⁴ livers. Only the amino-terminal portions of the Zn/Cd metallothioneins displaying sequence homology to the *Neurospora* copper metallothionein are shown. Identical residues are indicated by boxes.

What is the state of the copper bound in *Neurospora* metallothionein? Absorption and electron paramagnetic resonance spectroscopy of the freshly isolated protein suggest the copper to be present in the cuprous form. The lack of free cysteine residues and disulphide bridges as well as the broad featureless absorption around 250 nm are indicative of a copper(I)-thiolate binding mode. On the basis of these findings it is proposed that the model for *Neurospora* copper metallothionein is as shown in Fig. 3.

According to this model the net charge of the molecule is expected to be -1 at neutral pH, which is in line with the elution behaviour of the protein on DEAE-cellulose at pH 8.0. The structure is also supported on the grounds of model studies of Cu(I) complexes with sulphur-containing ligands¹⁵, as is the stoichiometry of ligand to metal of close to one.

What is the biological function of *Neurospora* copper metallothionein? Because copper is considered to be a rather toxic element for most organisms¹⁶ the protein could be involved in the detoxification of this metal as was discussed for metallothionein with respect to cadmium⁹. However, the large metal-binding capacity of *Neurospora* copper metallothionein also makes it a good candidate for an intracellular storage protein. This is supported by the results shown in Fig. 4*a*. The amount of copper metallothionein depends on the copper concentration up to approximately 100 μM and then levels off to reach a constant value up to 1 mM Cu in the culture medium. Above 2 mM the growth sharply declines, with a concomitant increase in free ionic copper intracellularly and a decrease in copper metallothionein.

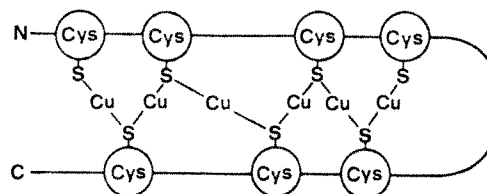


Fig. 3 Proposed model for *Neurospora* copper metallothionein.

These results clearly point to different functions of metallothionein in the copper metabolism depending on the amount of copper present in the culture medium. The complete uptake of the metal ion at low, presumably physiological concentrations of copper and the appearance of relatively large amounts of the metal ion intracellularly in the form of metallothionein (>40% of intracellular copper) is fully consistent with a storage function. On the other hand, the pronounced tolerance towards copper within a rather broad concentration range characterised by a constant copper metallothionein content suggests a detoxification function. At very high concentrations of copper (>3 mM) in the culture medium the toxic effects predominate; this is demonstrated by a sharp increase in intracellular free ionic copper and by a marked decrease in copper metallothionein.

As *Neurospora* copper metallothionein has an exceptionally high cysteine content the relationship between the available sulphur in the culture medium and the amount of copper metallothionein was also investigated (Fig. 4*b*). The data not only demonstrate a correlation between cellular copper uptake and appearance of metallothionein as pointed out above, but also show dependence of the two parameters on the sulphate

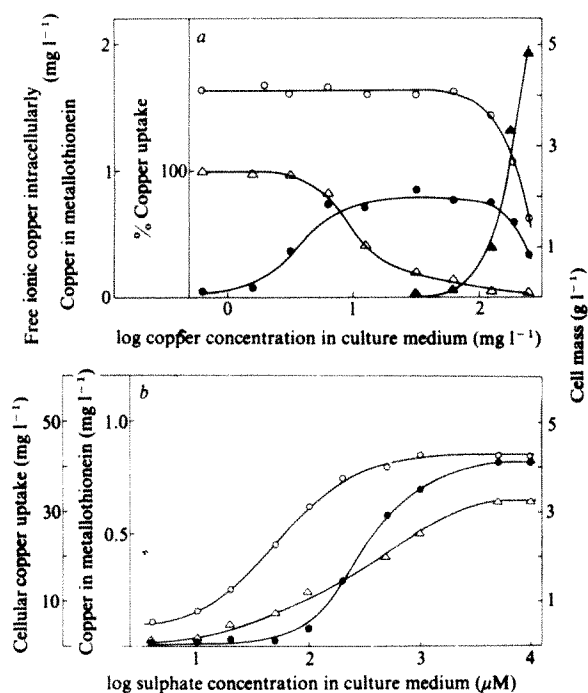


Fig. 4 *a*, The effect of copper added to the medium on the growth of *N. crassa* (○), the amount of metallothionein (●), the percentage of copper taken up from the culture medium (△) and the amount of free ionic copper (eluting at V_i on Sephadex G-50) (▲). Copper was added as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. Cells were collected after 72 h and processed as described in Fig. 1 legend. The total amount of copper taken up by the cells was determined by atomic absorption spectroscopy on samples wet-ashed at 120 °C for 2 h with an acid mixture made from a 2:1 ratio of concentrated nitric acid to 70% perchloric acid. *b*, The effect of sulphate added to the medium on the growth of *N. crassa* (○), the amount of copper metallothionein synthesised (●), and the copper uptake from the culture medium (△). Copper was added as $\text{Cu}(\text{NO}_3)_2$ at a concentration of 0.5 mM. Sulphate was added as $(\text{NH}_4)_2\text{SO}_4$. Cells were collected after 72 h and processed as described in Fig. 1 *a* legend.

levels in the culture medium. Further, the toxic effects of the copper as manifested by the decreased growth of the fungus are clearly related to the sulphate concentration. These results once more strongly suggest a function of copper metallothionein in copper detoxification.

Finally, copper metallothionein might serve the function of a metal donor to the active sites of copper-containing enzymes. This conjecture is supported by *in vitro* reconstitution studies with *Neurospora* apotyrrosinase and copper metallothionein. The complete reconstitution of apotyrrosinase (20 μM in 0.1 M sodium phosphate, pH 7.5) with a twofold excess of Cu^{2+} ions at 0 °C is a rather slow process (5–10 h); surprisingly, the treatment of apotyrrosinase with copper metallothionein in the same conditions is faster by almost an order of magnitude. As the concentrations of intracellular free ionic copper are extremely small in physiological conditions, it is tempting to ascribe to *Neurospora* copper metallothionein an *in vivo* metal donor function.

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Storage enhances chromosome damage after exposure of human leukocytes to mitomycin C

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Two of the distinctive differences between the mutagenic response of *Drosophila* sperm exposed to X rays and to alkylating agents, are: (1) an increase in the incidence of chromosomal structural changes with increasing storage time (days, or weeks) prior to fertilisation following treatments with chemical mutagens, but not X rays, and (2) the frequent occurrence of F_1 individuals mosaic for either mutation or chromosome structural change after chemical treatment, but not after X-ray radiation¹. Similar time-dependent increases in mutations and chromosome aberrations have also been reported for fungal spores² and plant seed root meristem cells³ exposed to alkylating agents, but no enhancing effect of storage could be demonstrated in confluent human fibroblasts exposed to an alkylating agent *in vitro*⁴. We show here that a storage effect can be demonstrated for sister chromatid exchange (SCE) and, to a much greater extent, for chromosome structural changes in human lymphocytes exposed to mitomycin C and stored for various periods before stimulation with phytohaemagglutinin (PHA). Moreover, mitomycin C-treated G_0 cells, which normally develop only chromatid-type aberrations, also yield chromosome-type changes after storage. This result implies a time-dependent alteration in the induced lesions following storage, and predicts a decreasing incidence of mosaicism with increasing storage time—a result that has indeed previously been reported for stored *Drosophila* sperm⁵.

The small lymphocytes in human peripheral blood are in a G_0 state and respond to PHA stimulation *in vitro* with the fast responding cells beginning DNA synthesis 24 h after stimulation and reaching mitosis at around 36 h (refs 6, 7). Following stimulation, cells may proceed through several mitotic cycles, with cycle times of around 16 h, over a period of a week or more in culture (unpublished data). Slow responding cells undergo a delayed transformation to a blast state such that they appear at their first mitosis many days after initial exposure to PHA. Fast responding cells are mainly T cells and slow responders contain a higher proportion of B cells⁸. To study the response of fast and slow developing cells to the induction of SCE and aberrations by mitomycin C, and to examine the effects of storage on induced damage, two types of experiment were performed.

For experiment 1, duplicate sets of blood cultures were set up in 10 ml RPMI 1640 medium (without serum and PHA) using 0.8 ml of heparinised whole blood from three healthy donors. One set was used as a control and the other treated with 6×10^{-7} M mitomycin C (Kyowa) for 1 h at 37 °C. The cells were then washed in medium and resuspended in fresh medium with serum, but without PHA, and incubated at 37 °C. Lymphocytes were stimulated to divide at 24 h intervals (from 0 to 9 days after treatment) by transferring to complete medium containing 20% fetal calf serum, 1% PHA (Wellcome), 1% glutamine, 100 units

penicillin, 100 µg streptomycin and 25 µM bromodeoxyuridine (BUdR). Stimulated cultures were kept in the dark at 37 °C and cells were collected at 72 h. Air-dried preparations were stained using the fluorescence plus Giemsa technique for differential chromatid staining⁹. With this protocol cells were kept for increasing periods, up to 9 days, in G₀ after mitomycin C treatment and samples of first and second division cells were available for up to 12 days after initial exposure to mitomycin C. Twenty second-division metaphases were analysed on each coded slide to determine SCE frequencies (Fig. 1). For chromosomal aberration incidence, first division metaphase cells which did not show differential staining were analysed from samples stimulated with PHA after 1, 5 and 8 days in culture and then fixed 72 h later (Table 1).

In the second experiment PHA and BUdR was added at the initiation of culture, the medium replaced with fresh complete medium every 72 h, and cells collected at daily intervals up to 7 days. The experiment was performed in two parts, and the SCE frequencies determined on coded slides are summarised in Fig. 2. The numbers of first division cells in late cultures were too small to give useful data on aberration frequencies.

The incidence of SCE in control cultures in experiment 1 showed no significant difference between donors or between culture times over a 9-day period. Mitomycin C exposure gave a typical eightfold increase in SCE levels over controls in cells stimulated over the period 1–4 days, but this was followed by a further significant increase (up to 100%) to a peak level at 6–8 days and a fall at 9 days. In cultures stimulated at time zero and sampled serially thereafter (experiment 2), there were no inter-donor differences and control samples showed a small increase in SCE with time in culture. Following mitomycin C treatment, there was again a rise in SCE frequency with time after exposure.

A trivial explanation of the increase in SCE with time after mitomycin C treatment would be that cells coming into their second mitosis around their sixth day in culture, whether stimulated at time zero and therefore 'slow' cells (experiment 2), or at day 4 and 'fast' cells (experiment 1), are more sensitive than other cells. This seems highly unlikely, since the samples scored

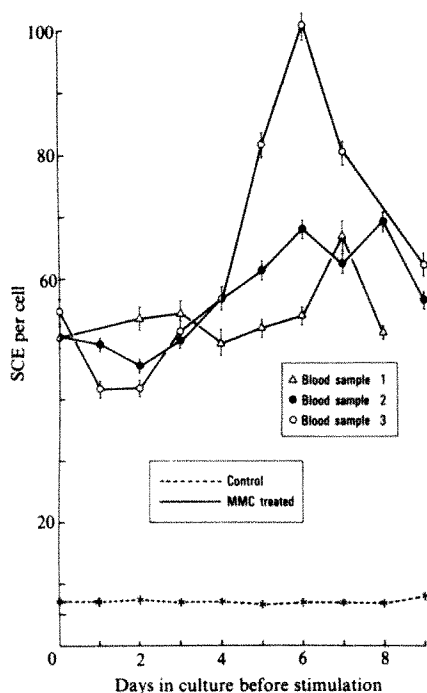


Fig. 1 Incidence of SCE (20 second-division cells per point) in lymphocytes from three donors exposed to 6×10^{-7} M mitomycin C for 1 h before culture and then stimulated with PHA at different times after treatment. Broken line, control; solid lines, mitomycin C-treated. Δ , Blood sample 1; \bullet , blood sample 2; \circ , blood sample 3.

Table 1 Chromosomal aberrations at the first division in cells treated with mitomycin C and then stimulated with PHA after various intervals in culture

Day of addition of PHA:	Controls			Exposure to mitomycin C*		
	1	5	8	1	5	8
Total metaphases analysed	150	150	150	150	140	100
Abnormal cells	4	5	4	16	19	40
Chromatid gaps	1	1	2	1	1	13
Chromatid breaks				4	3	7
Chromatid interchanges				1	5	9
Total chromatid breaks + exchanges (per cell)	0	0	0	0.033	0.057	0.160
Chromosome gaps† (isolocus)				2	2	6
Chromosome fragments†				3	7	11
Dicentric					2	2
Rings					1	1
Symmetrical intra- and interchanges						4
Total chromosome breaks + exchanges (per cell)	0	0	0	0.020	0.071	0.180

* Exposure (1 h) to 6×10^{-7} M mitomycin C at zero time.

† The categories of chromosome gaps and fragments may include some chromatid-type isolocus events.

at these times represent different cell populations. To confirm this point, in a third experiment we exposed cells either to PHA plus mitomycin C at time zero and sampled at 3 and 4 days, or to PHA plus mitomycin C at day 4 and sampled on days 7 and 8. The mean frequencies of SCE, and of chromatid aberrations, at days 3 and 4 did not differ from those found at days 7 and 8 (Table 2), so that the latter cells were not more sensitive than the former. There is, however, evidence of differences in response between B and T lymphocytes to the induction of SCE by BUdR, with the former showing a lower response¹⁰. Since B cells may predominate in very late cultures, this may account for a decline in SCE frequency at this time, but we have no evidence of a differential sensitivity of B and T cells to damage by alkylating agents. We conclude, therefore, that the mitomycin C-induced increase in SCE with time after treatment is a time-dependent phenomenon that is not a consequence of differential sensitivity, but is a true storage effect.

The data on mitomycin C-induced aberration frequencies in first division cells, that is, non-harlequin-stained metaphases, are summarised in Table 1. Two significant findings are evident: first, there is a marked increase in aberrations with time after mitomycin C treatment, the incidence following PHA addition on day 8 being seven times greater than on day 1; second, this increase is contributed to almost equally by chromatid- and chromosome-type aberrations. It is well established that proliferating somatic cells exposed to alkylating agents contain only chromatid-type aberrations at their first post-treatment mitosis. These aberrations, in which the unit of exchange or breakage is the single chromatid, are produced following exposure of cells at any stage in the cell cycle, and are a consequence of a mis-replication at sites of damage during the replication phase^{11,12}. In contrast, X-ray exposure of unreplicated chromatids in the G₀ stage yields chromosome-type aberrations, where breakage and exchange occurs prior to replication and the aberrant structure is itself replicated at the S phase¹³. Lymphocyte nuclei exposed to mitomycin C are in a G₀ state at the time of treatment and in all short term cultures the only aberrations seen at mitotic first

Table 2 Chromatid aberrations and SCE frequencies in cells exposed to 6×10^{-7} M mitomycin C for 1 h at zero hours or after 4 days in culture

Time of mitomycin C treatment	0 h		96 h	
Sampling time post-treatment	72 h	96 h	72 h	96 h
Chromatid aberrations per first division cell*	Controls	0 0.01	0 0	
	Mitomycin C-treated	0.05 0.08	0.05 0.11	
SCE frequencies in second division cells†	Controls	7.35 9.0	7.15 9.0	
	Mitomycin C-treated	53.1 57.8	51.9 55.5	

* 100 first-division cells analysed at each point.

† 40 second-division cells analysed at each point.

division in the first days following exposure are of the chromatid type. The presence of chromosome-type changes in first division cells when stored for periods of 5 to 8 days was therefore entirely unexpected.

Our demonstration of a large increase in chromosome aberrations and of a change in aberration type with increasing storage time following exposure to an alkylating agent, parallels earlier findings on *Drosophila* sperm. An aberration affecting a single chromatid in a fertilisation nucleus will segregate at mitosis to one of the daughter cells and may result in an individual mosaic for the abnormality, whereas a chromosome-type aberration, if viable, would be present in all descendent cells giving a whole-body mutant. Our result suggest that increased storage should result in a decreased proportion of mosaic mutants recovered and such a result has been reported for *Drosophila* sperm treated with triethylene melamine⁵.

We had previously shown that chromatid-type aberration frequencies in second division cells after exposure to alkylating agents are higher than in first division cells^{11,12}. This is consistent with the present findings and implies changes in the damaged DNA with time post-exposure. The interaction of mitomycin C with DNA is complex, although it is known that the semiquinone radical formed during its reduction in the cell binds very rapidly to DNA, particularly to the guanine moiety, and that the predominant site of alkylation is probably at the O^6 of

guanine¹⁴. Early changes in chromatid aberration frequencies may partly reflect single strand breakage due to chain instability following hydrolytic loss of alkylguanine, breakage arising either due to increased lability at the depurinated sites or to the action of an endonuclease II-type enzyme¹⁵. Mis-replication at sites of single strand damage may result in chromatid-type aberrations at replication, but chromosome-type aberrations require the presence of double-stranded scissions before replication¹⁶. Chain breakage following depurination may be a rare event, but chromosome-type aberrations may result from the coincidence of two single strand breaks that develop as secondary consequences of the alkylation that occurred many days earlier. Whatever the nature of the changes in lesions with time after exposure, these are more effective in increasing aberration frequency and result in only small increases in SCE.

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Partial nucleotide sequence of the 300-nucleotide interspersed repeated human DNA sequences

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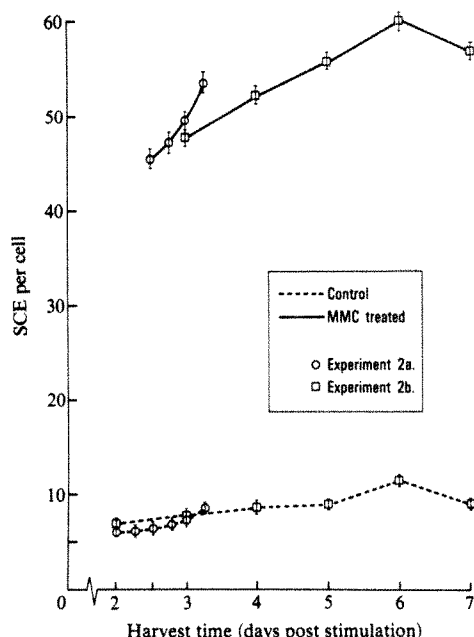
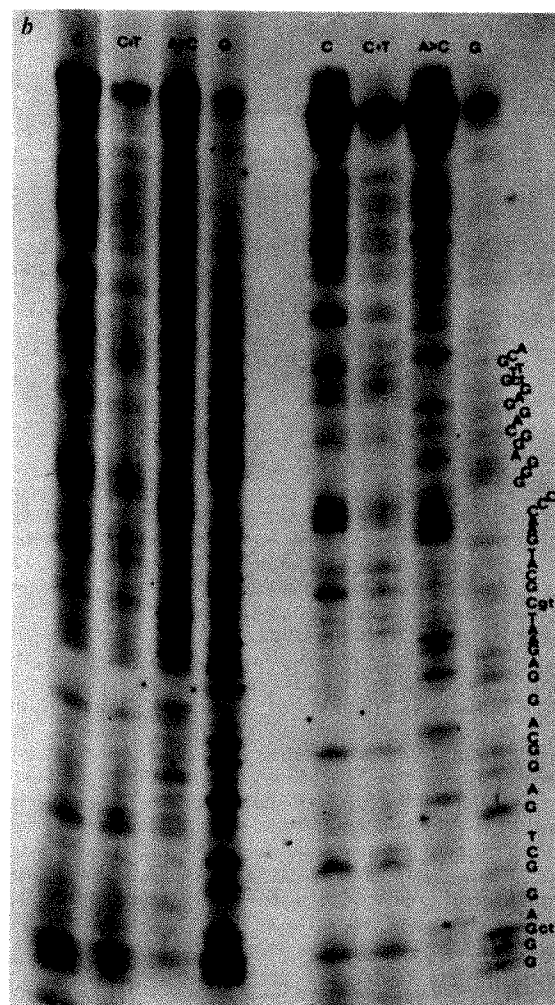


Fig. 2 Incidence of SCE (60 second-division cells per point) in lymphocytes from three donors exposed to 6×10^{-7} M mitomycin C for 1 h and then immediately cultured in the presence of PHA and serum. Broken lines, control; solid lines, mitomycin C treated. ○, Experiment 2a; □, experiment 2b.

In most eukaryotic genomes, including human, 300-nucleotide repeated DNA sequences are interspersed with longer (~1,000 nucleotide) single copy sequences^{1–3}. We have recently found that most 300-nucleotide interspersed repeats in human share a common site for cleavage by the restriction enzyme *Alu* and should be regarded as a single family of sequences⁴. We designate this as the *Alu* family of sequences. Similarly, most of the 300-nucleotide inverted repeated sequences, which are also interspersed with single copy DNA, share this same restriction site and belong to the *Alu* family⁴. There are approximately 300,000 members of this family of sequences, which together make up at least 3% of the human genome⁴. It is conceivable that individual members of the *Alu* family repeats share only very limited regions of homology, one of which happens to contain the restriction site for *Alu* and others which share the additional restriction sites reported here. In this case, members of the *Alu* family could be essentially different DNA sequences. DNA renaturation studies support the alternative view that members of the *Alu* family share extensive homology over the entire sequence length⁴. According to this view, individual members of the family could exhibit some divergence from the

As one control, we compare the fidelity of the sequence and its complement (Fig. 2). Complementary sequences were obtained as shown in Fig. 1a. In one portion of the proposed average sequence (positions -92 to -30) there are 11 differences in 62 assignments, which corresponds to an 18% sequence mismatching. In the other portion of the sequence (positions +12 to +69) there is 5% mismatching (3 differences in 56 assignments) between the sequence and its complement (Fig. 2). These values compare favourably to estimates from thermal stability studies which indicate an average of 12% mismatching in renatured interspersed repeated human DNA sequences¹.



As a second control on the accuracy of this sequence we have also determined the base sequence of some cloned members of the *Alu* family. Clones were constructed by ligating synthetic *Bam*HI linkers on the 300-nucleotide repeated sequences described above using a modification of a procedure used to clone sea urchin repeats⁷. These were propagated by insertion into the *Bam*HI site of the episome pBR322 and grown under P3, EK1 containment.

One clone, clone 8, was base sequenced in its entirety; another, clone 2, has been partially sequenced. The sequencing techniques used for this determination are summarised in Fig. 2, and we regard the assignments as being unambiguous. The sequences of these two clones are closely related to each other and to the average base sequence described above (Fig. 2). With the exception of a deletion in clone 8 at position 33, the overall sequence mismatching between clones 2 and 8 is 24% (27 differences in 113 shared sites). Twelve of these mismatches are clustered near position +75. A more extensive comparison of additional cloned sequences is required to establish whether

mutations are randomly distributed or clustered at certain preferred sites. It is especially interesting to compare the relative sequence fidelity of the two clones and the *Alu* family in the region shared by all three sequences, positions +3 to +69. There is 18% mismatching (11 differences in 61 sites) between clones 2 and 8 in this region. There is 22% mismatching (14.5 differences in 67 sites) between the *Alu* family and clone 8 and 21% mismatching (13 differences in 62 sites) between the *Alu* family and clone 2 in this shared region. The agreement between these estimates suggests that the average sequence proposed for the *Alu* family resembles the actual sequence as closely as any individual cloned member of the family. As previously noted, we expect 12% mismatching of renatured interspersed repeated human DNA⁵. Our representation of the *Alu* family therefore approximates the fidelity with which the sequence has been evolutionarily conserved.

Jelinek and co-workers have found that the repetitive double strand regions in HeLa cell heterogeneous nuclear RNA (hnRNA) have a very simple fingerprint⁸⁻¹⁰ dominated by six prominent oligonucleotides. This implies that repetitive hnRNA may contain one or at most a few distinct families of sequences⁸⁻¹⁰. In support of this conclusion, we find that five of the six oligonucleotides (oligonucleotides 1, 2, 3, 5, 6 reported by Jelinek *et al.*⁸) are present or approximately represented in the *Alu* family of sequences (Fig. 2). The remaining oligonucleotide 4 was later found to be a mixture of sequences and was not further characterised⁹. We find two sites in the *Alu* family which resemble oligonucleotide 4 and may be the composite source of that sequence (Fig. 2). The *Alu* family of repeated DNA sequences is sufficient to account for part if not all of the repetitive hnRNA sequences described by Jelinek and his colleagues⁸⁻¹⁰. As further evidence for this conclusion, Jelinek observed the same six prominent oligonucleotides in fingerprints of *in vitro* transcripts of inverted repeated DNA sequences⁹. As noted above, the 300-nucleotide inverted repeated sequences are members of the *Alu* family and should, as Jelinek observed, also contain these oligonucleotides.

We have restricted our description of this family of sequences to the human genome. However, work in progress in our laboratory indicates that a closely related family of sequences is found in all primate genomes (galago, monkey and human). Furthermore, Jelinek's observation of a single prominent oligonucleotide in the repetitive double-strand regions of hamster hnRNA is sufficient evidence to suggest that, in analogy to human, the hamster genome is also dominated by a single family of interspersed repetitive DNA sequences¹¹.

The biological function of this family of sequences is unknown. We and our colleagues have recently noted sequence similarities between a selected portion of the *Alu* family and several other RNA or DNA sequences, which are known or suspected to be involved in DNA replication, transcription control, and mRNA processing¹². Together these observations reinforce our belief that a family of DNA sequences which includes 300,000 highly conserved members interspersed throughout much of the mammalian genome, must have an important function.

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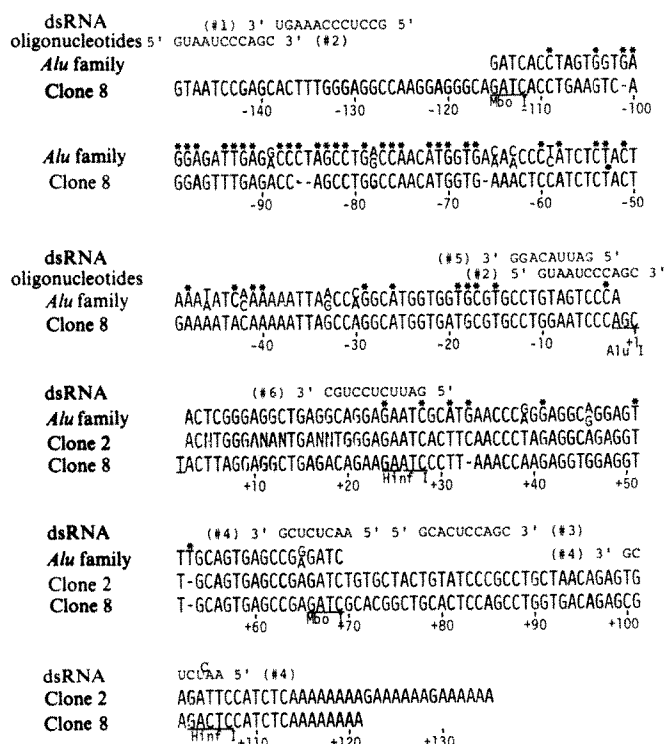


Fig. 2 The sequences of the *Alu* family and clones 2 and 8 are shown in large type. Since the ends of the sequence are not precisely defined, the sequence is numbered in both directions from the *Alu*I site. The method for determining the average sequence of the *Alu* family is described in the text and Fig. 1a. Asterisks designate sites at which the readings of either the sequence or its complement were ambiguous (see Fig. 1b). Positions at which the sequence and its complement did not agree are indicated by the presence of two bases. In the base pair fidelity calculations presented in the text these positions are assigned a value of 0.5 (that is, half mismatched). Clone 2 was cleaved at the artificial *Bam*HI linkers (see text), fill-in labelled at the 3' ends using α -³²P and reverse transcriptase and strand separated on a polyacrylamide gel. It was then sequenced by the Maxam-Gilbert technique⁶. Clone 2 was cleaved at the synthetic *Bam*HI sites, labelled at the 5' end with γ -³²P and polynucleotide kinase and cleaved with *Alu*I. The sequence between the right *Bam*HI site and the *Alu*I site was determined by the Maxam-Gilbert method. All the DNA sequences read from 5' to 3'. A deletion in one of the sequences compared to another is indicated as (-). The dsRNA oligonucleotides are shown in smaller type. The sequences of these oligonucleotides (1 to 6) are from the results of Jelinek and coworkers⁸⁻¹⁰. The polarity of the RNA strands is indicated in the figure.

MATTERS ARISING

Bragg intensities near structural phase transitions

IN a letter to *Nature* Åsbrink and Hong reported an increase of X-ray reflection intensity and profile widths at the low-to-high- V_3O_5 phase transition state¹. The oldest reference I know of to the increase of X-ray intensity near structural phase transitions is that of De Quervain² in 1944. The effect was observed in a ferroelectric material (KDP) of which large rather perfect crystals can easily be grown. As these crystals show severe extinction, any disturbance caused by a phase transition is liable to increase the reflectivity. Since then, this effect has been observed rather often near first-order phase transitions, and also with neutron or γ -ray scattering³. It has been used as a very accurate indication for the occurrence of the transition^{4,5} in a pressure cell. (We commonly use a series of crystals with various transition temperatures for accurate cryostat calibration.)

As this overshooting concerns integrated intensities, the width of the reflection profiles also increases. Various authors have used the information which lies in the variation near the transition of the profile shapes to characterise the state of the crystal near T_c . For example, Zeyen *et al.*^{6,7} have studied the reflection profiles of DKDP crystals during its ferroelectric first-order phase transition using high precision cryogenics⁸ and high resolution neutron diffraction techniques⁹. They investigated the spatial distribution of the phase mixing at T_c where both the paraelectric and the ferroelectric phases coexist in the crystal in a particular arrangement which actually minimises elastic and electrostatic (polarised domains) energies and depends on the structural change at T_c . Following their model the DKDP crystal forms a multi-layer of alternating paraelectric and ferroelectric sheets. It explains directly the overshooting intensity near T_c . The existence of these layers has in the meantime been confirmed directly by optical observation in a special cryostat¹⁰. Other systems giving different spatial arrangements have also been studied.

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ÅSBRINK AND HONG REPLY—We thank Zeyen for information on early discussions about intensity overshoot in connection with studies on KH_2PO_4 and KD_2PO_4 .

The possibility of phase mixing during the transition occurred to us; however, we had to discard it as several reflections did not even exhibit the profile widening at $t_T = 154.7^\circ\text{C}$ required by the difference in unit cell dimensions between the two V_3O_5 phases. (We made the comparative profile width measurements at 153 and 156°C for low- and high- V_3O_5 , respectively, and assumed that the entire change of unit cell dimensions between those temperatures took place at t_T .)

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Magnetostратigraphy, biostratigraphy and geochronology of Cretaceous-Tertiary boundary sediments, Red Deer Valley

LERBEKMO *ET AL.*¹ have presented some valuable radiometric data from an important continental sedimentary sequence which includes the Cretaceous-Tertiary boundary in Alberta. However, the palaeomagnetic data which they presented in their Fig. 2 show considerable scatter and do not provide a well defined polarity zonation.

Lerbekmo *et al.* correlate their magnetic polarity zonation from the Red Deer Valley with the magnetic polarity time scale. They note that the Cretaceous-Tertiary boundary, as recognised by marine biozonations, occurs near the base of anomaly 29. Lerbekmo *et al.* then concluded that the long normal polarity zone overlying the Cretaceous-Tertiary boundary in the Red Deer Valley, as recognised by dinosaur extinction and palynofloral zones, must correlate with anomaly 29. We consider this correlation to be circular reasoning based on the incorrect presumption that the Cretaceous-Tertiary boundary in the Red Deer Valley is synchronous with the Cretaceous-Tertiary boundary as determined by marine biozonations. This normal polarity zone in the Red Deer Valley could correlate with anomaly 28 or even anomaly 27. The magnetic polarity zonation in the Red Deer Valley does not show a convincing correlation to the magnetic polarity time scale. Thus, the conclusions reached by Lerbekmo *et al.*

based on their correlation are rather speculative.

In addition, Lerbekmo *et al.* proposed an alternative to our^{2,3} correlation between the San Juan Basin magnetic polarity zonation and the magnetic polarity time scale. They suggested that the normal polarity zone which we correlated with anomaly 29 should be correlated with a normal polarity interval between anomalies 29 and 30. However, there is no evidence for such a normal polarity interval in either of the two magnetostratigraphic sections at Gubbio, Italy^{4,5} nor in the magnetostratigraphic section at Moria, Italy⁶ nor in the marine magnetic anomaly record⁷. The palaeomagnetic data from the San Juan Basin provide a well defined magnetic polarity zonation which shows a strong correlation with the magnetic polarity time scale^{2,3}. We do not agree with Lerbekmo *et al.*'s reinterpretation of our data.

Lastly, we do not agree that there are "palaeontological discrepancies between the New Mexico, Alberta, and Gubbio, Italy sections with respect to the Cretaceous-Tertiary boundary". As we pointed out², the Cretaceous-Tertiary boundary is defined by extinction of marine invertebrates but is recognised in terrestrial sedimentary sequences by the last occurrence of dinosaur fossils. These two biological events need not have occurred synchronously and marine/non-marine intertonguing relationships do not provide enough precision to test for global synchronicity of this geological-time boundary. However, magnetostratigraphy does provide a possible technique for determining the temporal relationship of dinosaur extinction and marine invertebrate extinctions marking the Cretaceous-Tertiary boundary. Thus our magnetostratigraphic data which indicate a lack of synchronicity between dinosaur extinction in the San Juan Basin and the Cretaceous-Tertiary boundary at Gubbio, Italy do not constitute a palaeontological discrepancy as implied by Lerbekmo *et al.*

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LERBEKMO *ET AL.* REPLY—Butler and Lindsay have focused attention on some of the problems in magnetostratigraphic correlation. They point out that our palaeomagnetic data show considerable scatter and do not provide a well defined polarity zonation. This reiterates the statement in our original article. Nevertheless, there is a stratigraphic zonation present—the data are not randomly scattered. We believe the key to interpretation of this type of data is the recognition of the importance of viscous and/or chemical overprinting; nature has not provided ideal material and we have attempted a rational interpretation following the reasoning of Hillhouse *et al.*¹. A closely comparable study involving the same problems, and following essentially the same scheme has been reported by Brown *et al.*². We are convinced that a real pattern of normal and reversed polarity emerges from our data, although some overprinting may still be present.

Butler and Lindsay suggest that we have used circular reasoning in our anomaly matching, which we have not. This can probably be attributed to the limited palaeontological background information contained in our original article. In fact, we believe that our correlation of the Red Deer Valley section with the Gubbio section in Italy is unambiguous for the following reasons.

The palynomorphic change which takes place just above the Nevis coal seam in Alberta³ and at the Hell Creek (Lance)—Fort Union contact in Montana and Wyoming³, also takes place at the Hell Creek—Ludlow (Lignite Beds) contact in North Dakota in the type area of the Cannonball Formation⁴. The Ludlow there is 5–20 m thick and is overlain by the marine Cannonball which reaches a thickness of 125 m in the subsurface at Garrison Dam⁵. Foraminifera recovered from the Garrison Dam core place the entire formation in the *Globigerina edita* zone, which is the lowest Palaeocene foraminiferal zone and is equated⁵ to the *Globorotalia pseudobulloides* zone plus the thin *Globigerina eugubina* zone (which occur in the Gubbio section). The Cannonball Formation spans the *Globorotalia pseudobulloides* zone and is separated from the Cretaceous–Tertiary boundary below, as defined by palynomorphs, by about 20 m of Lignite Beds (=Ludlow)^{4,5}. The *Globorotalia pseudobulloides* zone at Gubbio encompasses part of anomaly 28, all of anomaly 29 and part of the reversed zone between anomalies 29 and 30 (ref. 6). Therefore, if the palynomorphic change equated with the Cretaceous–Tertiary boundary in Alberta, Montana, North Dakota and Wyoming³ is essentially synchronous throughout this area, as believed⁴, this palynomorphic boundary and the highest occurrences of dinosaurs are in the same reversed zone in which the foraminiferal Cretaceous–Tertiary boundary takes

place in Italy (that is between anomalies 29 and 30).

The anomaly matching by Butler *et al.*⁷ and Lindsay *et al.*⁸ is not infallible; whether or not the results summarised in Fig. 2 of ref. 8 constitute a “strong correlation with the magnetic polarity time scale” is debatable. The problem of ‘extra’ polarity intervals arises. In their comment above Butler and Lindsay question the validity of an additional normal interval between 29 and 30. However, in Fig. 2 of ref. 8 two ‘extra’ polarity intervals are shown which do not appear in the seafloor polarity record⁹. We expect that detailed palaeomagnetic studies of this type will reveal short magnetic zones not now recognised in the seafloor anomaly pattern, but much further work will be required to separate these from fictitious overprinted intervals.

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British Tertiary Igneous Province probably not associated with East Greenland lavas

IN their recent article Carter *et al.*¹ discuss the source regions of continental basalts extruded round the North Atlantic in the early Tertiary. They made the common assumption that the British Tertiary Igneous Province (BTIP) was formed as a result of the rifting which led to the separation of Greenland from Europe. Their reason for this assumption was presumably that the formation of the BTIP was close in space and time to the

rifting. However, this similarity may be more apparent than real and be misleading by concealing significant differences, summarised below.

Carter *et al.* give the duration of activity in the BTIP as 66–50 Myr ago. Although such a spread of dates—and greater—can be found in the literature, note that the province has proved particularly difficult to date accurately, and many published dates are inconsistent with the stratigraphy, for example K–Ar dates for Faeroes² and Mull dykes³. If only the most reliable dates are used, which means chiefly ⁴⁰Ar/³⁹Ar plateau and Rb/Sr isochron ages, most activity is found to have occurred about 60 Myr ago (Table 1). (All dates quoted here have been adjusted to the decay constants and so on of Steiger and Jäger⁴.) Only Lundy is clearly younger.

The time of opening of the North-east Atlantic is not yet agreed, but is usually assumed to have just preceded the formation of magnetic anomaly 24. The age of this anomaly is deduced by calibrating the anomaly pattern of Heirtzler *et al.*⁵ at various points; because the spreading rate was probably not uniform this leads to a range of estimates for the age of anomaly 24. However, polarity time scales which have been calibrated in the Palaeocene^{6–8} assign it ages of 50.3, 53.4 and 54.4 Myr, respectively. Although the Greenland basalts must be somewhat older than this, it seems unlikely that they are contemporaneous with the BTIP, with the possible exception of Lundy.

Another difference is that activity on the British side of the North-east Atlantic—to be occurred in discrete areas. Most of these areas lie roughly on a line joining the Faeroes to Lundy (St Kilda and Rockall lie well to the west) which diverges at an angle of ~55° from the margin of the Atlantic, according to the reconstruction of Bullard *et al.*⁹. Thus Arran, for example, is a perpendicular distance of ~300 km from the margin, while Lundy (the part of the BTIP most likely to be contemporaneous with the rifting) is over 500 km distant. In contrast, the Greenland activity lies within 150 km of the margin.

The East Greenland coast has a flexure and associated dyke swarm. In contrast, the BTIP has no flexure and the regional dyke swarms of the separate areas, although roughly parallel to each other, are *en echelon*; these swarms are not what

Table 1 ⁴⁰Ar/³⁹Ar plateau and Rb/Sr isochron dating

Area and rock	Method	Age (Myr)	Ref.
Arran: Northern Granite	⁴⁰ Ar/ ³⁹ Ar plateau	60.4 ± 0.6	10
Mournes: granite	⁴⁰ Ar/ ³⁹ Ar plateau	59.7 ± 1.6	10
Mull: dyke cutting Loch Bà Felsite	⁴⁰ Ar/ ³⁹ Ar plateau	60.3 ± 3	10
Mull: centre 3 granite	Rb/Sr isochron	58.2 ± 2.5	11
Antrim: basalts	K/Ar isochron	~60	12
Lundy: granite	Rb/Sr isochron	53 ± 2, 55 ± 3	13
Lundy: granite	⁴⁰ Ar/ ³⁹ Ar plateau	53.4 ± 1.4	14

would be expected along, or beside, a simple tensional split but rather, say, what would result from a southward movement of Ireland relative to Great Britain.

Most of the activity of the BTIP probably preceded the opening of the North-east Atlantic and the Greenland activity by several million years; it does not occur along the margins of the rift, and occurred in a stress field that does not seem to be related to that of an opening ocean.

These observations do not affect the conclusion of Carter *et al.*, that there is no evidence for an undepleted mantle source-region for any of the basalts they discussed. However, their assumption that the BTIP is simply due to the formation of the North-east Atlantic oversimplifies our understanding of how the BTIP formed. Here it is relevant that they point out that the BTIP may be anomalous as there may have been "overall higher temperatures in the lower crust in North-west Scotland at the time of basalt eruption than elsewhere in the North Atlantic Tertiary Province".

Perhaps it just was not part of this province!

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Cubomedusae belong to the class Cubozoa, not Scyphozoa

IN their report on the neurophysiology of *Carybdea rastonii* Haacke (a cubomedusa), Satterlie and Spencer¹ emphasised that "... properties of the neuromuscular system ... clearly show it to be of the scyphozoan type ...". They also name the Cubomedusae as an order within the class Scyphozoa according to Haeckel's taxonomic scheme². Since the discovery of the first polyp of a cubomedusa³, Werner^{4–7} and Chapman^{7,8} have made extensive studies of the anatomy, behaviour, life history and systematic position of members of this group. The results of these studies led Werner to give

the Cubomedusae the rank of a class (Cubozoa)^{4,5}. This conclusion was supported by Chapman's study of the microanatomy of a cubopolyp⁸.

The principal factor which has led to the new classification is the structure of the polyp. Those cubopolyps which have been studied (*Tripedalia* and *Carybdea*) differ from polyps of the class Scyphozoa in that they lack, among other features, the gastric septa and tetramerous symmetry which are diagnostic of scyphopolyps^{5,6,8–10}. The cubopolyps share radial symmetry, lateral budding, capitate tentacles and other important features with hydrozoan polyps and also possess unique features^{5,6,8}. The neuromuscular system (which Satterlie and Spencer¹ equate with that of the Scyphozoa), like the features mentioned above, clearly illustrates the mixture of unique, scyphozoan-, and hydrozoan-like features in the Cubozoa. For example, it has long been known that cubomedusae possess a marginal nerve ring^{2,9}, like the hydromedusae but unlike the scyphomedusae. A unique feature of the Cubozoa is the presence of an ectodermal-endodermal nerve-ring pair in the polyp⁸. Although Satterlie and Spencer¹ cite the paper by Werner *et al.*⁶ on the neuromuscular system of a cubopolyp, they discuss in their own article only the medusa of *Carybdea* and only scyphozoan-like features of the medusa.

When one is dealing with organisms with a complex life history, such as coelenterates, one must bear in mind all stages of the life cycle if one wishes to draw conclusions about taxonomic position. Satterlie and Spencer's¹ fascinating findings on the neurophysiology of *Carybdea* do not support the retention of the Cubomedusae in the class Scyphozoa but do provide further support for Werner's^{4,5} theory that the Cubozoa are intermediate between the Scyphozoa and Hydrozoa and that the Scyphozoa are the class most similar to the ancestors of the three groups.

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SATTERLIE AND SPENCER REPLY—
Our article was not intended to give

definitive evidence for the taxonomic position of the Cubomedusae. However, judged by the neuromuscular properties of the swimming system of *Carybdea*, the medusae strongly resemble the scyphomedusae. This similarity is so convincing as to make it unnecessary to create a new class, the Cubozoa, to accommodate any unique features. In addition, the structure and function of the swimming system of *Carybdea* is unlike any known hydromedusan system.

The one cubomedusan feature frequently considered hydrozoan-like, the subumbrellar nerve ring, is not structurally or functionally similar to the marginal nerve rings of hydromedusae. In the latter, swimming pacemakers are distributed throughout the inner nerve ring, as an electrically coupled network of 'giant' neurones. In both cubomedusae and scyphomedusae, the swimming pacemakers are restricted to the rhopalial. Transmission of pacemaker output throughout the subumbrellar muscle sheet in both cubomedusae and scyphomedusae is through networks of large bi- and tri-polar neurones, making up subumbrellar nerve nets. Although subumbrellar nerve nets are present in some hydromedusae, the subumbrellar muscle cells show widespread intercellular electrical coupling, and in some cases, make up a conducting musculo-epithelium. Ultrastructural evidence argues against such electrical coupling in cubomedusae. Each swimming contraction of hydromedusae is an all-or-none event, accompanied by an all-or-none action potential in the muscle sheet. In comparison, both cubomedusae and scyphomedusae exhibit graded muscle contractions, and at least in *Carybdea*, graded muscle potentials. As this short comparison points out, not only is there a lack of similarity between the hydromedusan and cubomedusan swimming systems but also a lack of originality on the part of the cubomedusae, as all features are distinctly scyphozoan-like.

As Leonard points out, most of the evidence for proposing a new class comes from work on the structure of cubopolyps. It is difficult to determine whether some of the structural characteristics of cubopolyps, such as the lack of tetramerous symmetry and gastric septa, are primitive features or secondarily derived from a true scyphozoan ancestor. Cubomedusae exhibit structural specialisations in both stages of their life cycle; however, at least in the medusa generation, these specialisations are morphologically and physiologically consistent with the scyphozoan line.

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Enhanced excision of O^6 -alkylguanine in rat liver by pretreatment with acetylaminofluorene

AS recently reported by Buckley *et al.*¹, a diet containing 0.06% 2-acetylaminofluorene (AAF) established in rats a condition in which the liver of the animals had an increased capacity for removing O^6 -methylguanine from DNA produced by a single dose of dimethylnitrosamine (DMN). These observations are open to many interesting interpretations. However, the authors concentrated exclusively on the concept of induction of specific repair enzymes by the presence of damage from AAF adducts. This interpretation does not pay due attention to the biological state of the cell population in the liver at the time of the investigation.

Chronic ingestion of a liver carcinogen such as AAF will stimulate the liver into hyperplasia. Although the authors specifically excluded animals that had nodular livers, it is likely that livers of all animals at the end of this chronic treatment will contain an increased proliferative population^{2,3}. Passage between the non-cycling and proliferative phases of a cell population alters the activity of many enzymes involved in DNA replication and repair. Peripheral lymphocytes stimulated to proliferate by phytohaemagglutinin increase their capacity for repair⁴ and synthesise a specific repair enzyme, uracil glycosylase⁵. In the rat liver, DNA polymerase β , which may participate in excision repair, increases during regenerative hyperplasia following partial hepatectomy⁶. The repair systems do not all respond equally, because the system that removes O^6 -methylguanine increases during liver hyperplasia after chronic treatment with DMN, whereas that for removing N^3 -methyladenine or N^7 -methylguanine does not⁷. Excision repair seems to be particularly responsive to the proliferative state of a cell population⁸⁻¹⁰. One interpretation of the observations of Buckley *et al.*¹ may therefore simply be that they represent the differences between the repair capacity of a quiescent relative to a proliferating cell population. The increased repair of O^6 -methylguanine would not relate specifically to signals from damaged sites inducing the synthesis of new enzymes, but rather to a more generalised consequence of stimulation of proliferation.

Buckley *et al.* imply that exposure to AAF induces the repair of lesions formed by an unrelated carcinogen, DMN. The lesions formed by AAF and the O^6 -methylguanine produced by DMN are not, however, as different as might be expected if one considers the pathways by which they are repaired. Both kinds of lesion can be repaired by the nucleotide excision repair pathway¹¹. This system removes pyrimidine dimers produced by UV light and is regulated by the genes

associated with the human disease xeroderma pigmentosum (XP)¹². The autosomal recessive mutations in XP eliminate the capacity to remove O^6 -methylguanine, O^6 -ethylguanine, pyrimidine dimers, and the lesions produced by *N*-acetoxy-AAF¹³⁻¹⁶. Interestingly, O^6 -methylguanine is not repaired by the nucleotide excision repair system in *Escherichia coli*, although it is in mammalian cells^{15,16}, suggesting that prokaryotic systems may be very poor models to use in the interpretation of observations of O^6 -methylguanine in mammalian cells.

Buckley *et al.* cite evidence for the induction of post-replication repair by *N*-acetoxy-AAF-treated Chinese hamster cells¹⁷, but that observation only demonstrated alterations in semi-conservative DNA replication confined to the first few hours after an acute dose. Those alterations involved the growth of nascent DNA chains, and did not involve any change in the repair of lesions on the parental DNA; also, they have been interpreted elsewhere in a manner that does not invoke the induction of any system¹⁸.

Therefore, we suggest that a change in the excision capacity of liver by pretreatment with AAF more probably reflects the production of a spectrum of enzymes associated with cell proliferation, especially for DNA replication and repair, than the specific induction of unique repair enzymes. Moreover, we should also point out an interesting implication of observations of Buckley *et al.* in relation to the role of O^6 -methylguanine as a critical lesion for carcinogenesis by alkylating agents¹⁹. When rats were given the same feeding regimen of AAF and DMN, the subsequent yield of hepatocellular carcinomas reached nearly 100%, whereas a single dose of DMN given alone produced no liver cancers²⁰. Thus, increased repair of O^6 -methylguanine correlates with increased susceptibility to hepatocarcinogenesis, whereas the opposite correlation prevails in the neonatal rat brain, in which decreased repair of O^6 -methylguanine correlates with elevated carcinogenesis²¹. These contrasting correlations indicate that any attempt to ascribe a unique, definite role to O^6 -alkylguanine in carcinogenesis is premature; the role of this product and its repair are not yet fully understood. At the very least, the balance between DNA repair and DNA replication in each experimental situation needs to be carefully considered.

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BUCKLEY, O'CONNOR AND CRAIG
REPLY—Although more detailed information will be available when a fuller report is published on the basis of our recent study¹, two comments should be made on the matters raised by Cleaver and Kaufmann. First, we did not intend to imply that our interpretation rested exclusively on the induction of specific repair enzyme systems. Had that been the case we would not have used the phrase "general repair mechanism". Second, with regard to the final statement on the role of O^6 -alkylation of guanine in DNA, we agree that an increased capacity for the repair of O^6 -methylguanine in liver DNA correlates with a high susceptibility to hepatocarcinogenesis in the case of rats chronically exposed to dimethylnitrosamine. Indeed, this may also be the case in our experiments with rats exposed to AAF, although the situation has not been evaluated in our own Wistar rats. While recognising that the role of O^6 -alkylguanine in carcinogenesis is not fully understood it seems unreasonable to consider just two experimental situations (increased O^6 -methylguanine repair after pretreatment and the persistence of O^6 -alkylguanine in the brain of neonatal rats with respect to tumour formation) and not to take into account the considerable information now available (reviewed in refs 2-5). In fact, from reports referred to in these reviews it is clear that careful consideration has been given to the relevance of DNA repair and DNA replication.

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BOOK REVIEWS

Meeting the challenge of eukaryotes

Kenneth Burton

LIKE the well-known first volume that appeared over five years ago (reviewed in *Nature* 252, 145; 1974), this book is a selection of topics written by different authors for the advanced student and aimed at the niche between basic textbooks and specialist reviews. The earlier volume was rather hefty and had mainly general or prokaryotic topics; the new one is of a more reasonable size and its fifteen chapters illustrate the growing confidence of molecular biologists to approach the many challenges posed by eukaryotes.

The authors have generally avoided the temptation to be too comprehensive and sometimes linger to analyse controversies, unanswered problems or the limitations of experimental techniques. I doubt if there can be any biochemist already so knowledgeable over the whole field that he could not find a good number of novel and intriguing topics in the book. Yet no chapter should be beyond the reach of a final-year honours undergraduate, apart from the theory in Blake's article on X-ray crystallography which needs particularly good mathematical preparation. Without it, however, much can still be gleaned including an illuminating discussion of structures derived from single crystal X-ray diffraction. The full appreciation of Tipton's account of the kinetic properties of allosteric and co-operative enzymes also needs a mathematical grasp that is regrettably beyond that of some undergraduate biochemists.

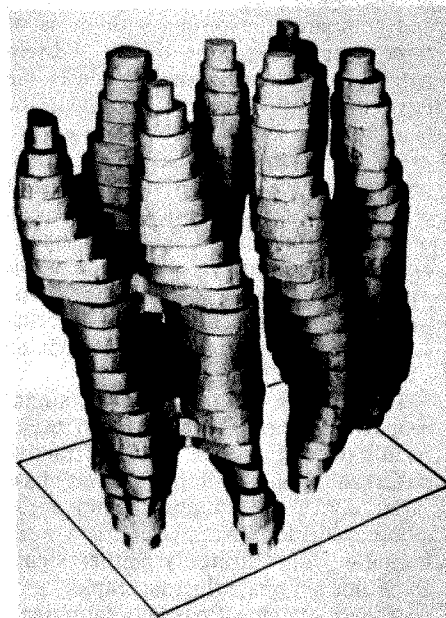
Several other chapters are also of general application to many areas of biochemistry. Nigel Brown knew he had a difficult task to review nucleic acid sequencing, for here the speed of biochemical innovation outpaces the leisurely tread of book publishing. His article was splendid when it was written in

Companion to Biochemistry. Vol. 2. Edited by A.T. Bull, J.R. Lagnado, J.O. Thomas and K.F. Tipton. Pp. 490. (Longman: Harlow, UK, 1979.) £13.95.

1977 but the dideoxynucleotide chain termination method for DNA sequencing and rapid methods for RNA sequencing were not originally included and only get a very brief mention in an addendum. Where space must represent cost, it is surprising to see nearly a page given to the formulae of carbodiimide modification of uracil as well as six pages to the complete sequence of ϕ X174 DNA, however splendid and important the elucidation of that sequence may have been. Glover and Rigby give a lively and stimulating account of gene cloning and recombinant DNA procedures, and how they can be used. As any *Nature* reader will know, this field is also moving forward at a rush aided by the fast methods of DNA sequencing. Again, important applications and developments since the middle of 1977 are not discussed fully though some are mentioned in the inevitable addendum, with only four citations from 1978 and, of course, none from 1979. Thomas clearly expounds the more incisive arguments about the basic molecular structure of chromatin.

Hardy's chapter on ribosomes succeeds in being most useful for the non-specialist but there are other more detailed (and often turgid) reviews on ribosomes elsewhere. Campbell contributes a lucid, well-argued article that is a joy to read on the biosynthesis of proteins destined for secretion. Rumsby's chapter on membranes is less successful: the prose style is guaranteed to switch off all but the most ardent student (for example, "A

consideration of the phase properties of complex lipids is fundamental in understanding current views on the structure of lipids in biological membranes. On heating complex lipids exhibit an intermediate mesomorphic ..."). Moreover, there are unfortunate mistakes in the calculation of protein/lipid ratios in Table 6.3.



Hales, Elder and Davies have chosen to comment on general aspects of clinical biochemistry and the training of clinical biochemists as well as to discuss inherited abnormalities of enzyme function and the use of immunological assays, especially in endocrinology. These discussions are excellent, apart from the lack of up to date references for the last topic.

The remaining chapters are in more specialised fields, such as cell-surface receptors and neurotransmitters (Michell), macromolecular aspects of nervous communication (Lagnado and Beale), complement (Reid), photosynthesis (Gregory) and vision (Voaden). Several of these authors offer aperitifs of relevant background biology that whet the appetite for their main course. Laskey writes most lucidly on the biochemical basis of cell specialisations in early embryonic development. He emphasises DNA

replication, transcription and protein synthesis with an analytical approach that is free from unprofitable speculation. Intermediary metabolism of animals is not given a chapter and plants get only one, on photosynthesis.

I am sure that this book will be read, studied and enjoyed with much profit by advanced students and teachers of biochemistry, even though some chapters are already somewhat obsolete, so raising the question whether quicker ways of production cannot be found for this type of

material. Although there is no use of colour, the presentation is generally good; the type is clear, and there are many good line diagrams and a full subject index. Photographic material is well-chosen, though the quality of reproduction might have been better. The cost per page is not unreasonable by comparison with other publications of similar purpose, such as *Essays in Biochemistry*. □

Kenneth Burton is Professor of Biochemistry at the University of Newcastle upon Tyne, UK.

Ocean sound speed fluctuations

John A. DeSanto

Sound Transmission through a Fluctuating Ocean. Edited by S. M. Flatte. Pp. 299. (Cambridge University Press: Cambridge, 1979.) £17.50.

THE book is a very well-edited collection of results arising out of the Jason group of the Advanced Research Projects Agency. It presents their theoretical treatment of the effects of a particular model of ocean sound speed fluctuations (due solely to internal waves) on acoustic propagation, and compares the results with those of three experiments, one in the Western Atlantic (Eluthera-Bermuda), one in the Eastern Atlantic (Azores), and one in the North-eastern Pacific (Cobb Seamount).

Both deterministic and statistical properties of the ocean differ from those of other media. Deterministically, the ocean is refractive in depth (unlike atmospheric optics). Statistically, ocean fluctuations differ from those of homogeneous isotropic turbulence, for example, by being anisotropic, inhomogeneous and by having a different spectral function.

The effects on an acoustic signal propagating through the random ocean region depend on the size (diffractive effect) and strength (simply, the rms phase variation) of the random sound speed inhomogeneities. If the region is a weak scatterer ('unsaturated') neither signal amplitude nor phase fluctuate rapidly. For a stronger scatterer ('partially saturated') the phase begins to fluctuate rapidly, and for a very strong scatterer ('saturated') both phase and amplitude fluctuate rapidly. Also, the longer the propagation range and/or the higher the frequency the more the saturation.

The book contains several parts. Part I is on the ocean environment (ocean structure, planetary waves and eddies, and linear internal waves), and is an excellent introduction to the physics of the ocean

and the internal wave fluctuation model used throughout. The fluctuations are thus modelled as arising solely from that spectral window of ocean variability bounded below by the Coriolis (inertial) frequency and above by the Väisälä (buoyancy) frequency.

In Parts II, III and IV, the theoretical methods of sound transmission are developed and interrelated. Path-integral methods (useful in the saturated region), the Rytov approximation (unsaturated region), and the parabolic approximation are all developed, with particular attention paid to ray-theory methods. Of particular use is the discussion of the statistics of acoustic signals in regimes of different saturation.

Do internal waves account for acoustic fluctuations? Part V compares the theoretical predictions to the three experiments. With this extensive and careful development one rather tends to expect a smashing success. It isn't so. The results are mixed. For the (saturated)

Eluthera-Bermuda experiment the rms phase rate and Cartesian signal statistics are predicted well, phase and intensity spectra poorly. For the Cobb Seamount experiment (unsaturated) the internal wave model doesn't account for the measured intensity fluctuations. For the Azores experiment (partial saturation) the agreement is generally good.

My opinion is that the mixed results should not detract from this well written book. It contains a great deal of information, a consistent focused methodology, and the starting point for much additional work. It is suited to an advanced graduate course; and its balance of theory, computations and experimental comparison can serve as a model for extension to the effects of other ocean spectral regimes on acoustic propagation. □

John A. DeSanto is a research physicist at the Naval Research Laboratory, Washington, DC.

Geothermal energy

E. R. Oxburgh

Geothermal Energy. By H. Christopher H. Armstead. Pp. 357. (E. and F. N. Spon: London, 1978.) £10.50.

OF THE 'alternative energy' resources attracting attention in the early seventies at the time of the rapid rise in world oil prices, geothermal energy is probably the only one for which the expectations are better today than they were five years ago. On the other hand, it must be admitted that at that time expectations were rather low, certainly in Britain.

The exploitation of geothermal energy involves extracting water from sufficiently deep in the ground that it arrives at the surface hot enough for useful heat to be

obtained from it. In geothermal prospecting the aim is to identify places where subsurface temperatures are abnormally high and where, at the same time, the rocks are sufficiently permeable to permit the circulation of water through them. Although in principle any desired temperature up to about 250°C could be reached by deep drilling, in many places this value would be reached only at depths of 10-12 kilometres, close to the depth limits of present drilling technology. Drilling costs are so high, however, that at present the economic viability of a geothermal prospect depends on whether water at useful temperatures can be obtained from depths of less than about 4 kilometres. Of the geothermal power in use today (providing about 0.25% of the world's electrical energy) the greater part is derived from much shallower depths than this; in volcanic areas it is often possible to work with holes of the order of a kilometre deep.

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In *Geothermal Energy*, the author touches on every side of the subject. He is clearly an enormous enthusiast and it is his enthusiasm that carries the reader through the book. Indeed in some ways it may be viewed as part handbook and part evangelical tract for the cause of geothermal energy!

The book contains 357 pages of which about 60 are devoted to the general nature of geothermal areas and their geological setting, about 30 to geothermal exploration and drilling, about 160 to the techniques of exploitation and heat extraction at the surface, and about 30 to possible and actual applications of geothermal energy. The remainder is taken up with the index and bibliography (together 25 pages), wide-ranging discussions of problems of pollution, future patterns of world energy usage, and a host of other topics.

There is at present no other single book available with the scope of the present volume, although much of the material is to be found dispersed in an intractable diversity of technical publications. For this reason, if for no other, the book is a valuable one and will be widely used. The author adopts a no-nonsense, down-to-earth, practical approach to the subject which in places is excellent, but

occasionally leads to inaccurate oversimplification. He is at his best when discussing the practical problems and techniques of geothermal field exploitation. Elsewhere he may be idiosyncratic (for example, on page 46, "meteoric waters . . . is merely a 'snob' term for rainwater" — apparently connate water is acceptable); imprecise (for example, on page 85, "... temperature rises less rapidly than depth increases."), or even misleading (on page 44, "... and because of gaping cracks penetrating deeply into the mantle . . ."). This reviewer also feels that there is little point in devoting several pages to fanciful calculations of the total heat content of the Earth, even if the author does at the end emphasise that they are irrelevant to any assessment of exploitable geothermal reserves.

This is a valuable contribution to the literature of the subject. It should be on the shelves of scientific libraries. Its strength and weakness is that it is written by a slightly breathless, uninhibited enthusiast who perhaps oversells his product. □

E.R. Oxburgh is a Lecturer in the Department of Mineralogy and Petrology, University of Cambridge, UK.

How to put your laboratory in order

R.C. Holloway

Laboratory Organization and Management. By F. Grover and P. Wallace. Pp.241. (Butterworth: Sevenoaks, UK.) £5.95.

To many readers of this volume, the thought that they are 'managers' may well be anathema, yet, whether we like it or not, laboratories will no doubt become more organised, like most other institutions. The authors press the point that good management (but not bureaucracy) is a deciding factor between a successful and a poorly organised laboratory. In the present financial climate no doubt this is relevant.

Two chapters, on laboratory planning and service departments, encompass a wide range of subjects in a comparatively short space. There are many practical and useful hints but one is left with the distinct feeling that one has passed over rather too much, too quickly. Certainly, the style tends towards the turgid, with lists sometimes posing as sentences.

Most of the rest is devoted to the fine detail of management. Nearly 30 pages are concerned with the selection and management of staff, from the moment a vacancy

occurs until the time when the appointee leaves the establishment. Choosing the right applicant is undoubtedly a critical matter and mistakes are often made through inexperience, but this chapter reads rather like a miniature course for personnel officers. Store management and laboratory administration are followed by management technique and function. Here we are introduced to forecasting, planning, organising, motivating, controlling and communicating, in considerable detail. As would be anticipated from the authors, the chapter on safety is a model survey of safety organisation and the hazards involved.

Of errors and omissions there are very few. A reference to Dr Hughes' booklet (p.145) on *Design of Radioactive Laboratories* refers to "ionic" instead of "toxic" substances and there is a strange juxtaposition of litres per hour and gallons per day on p.27. A word of warning on present doubts about the safety of biological recirculatory cabinets (p.174) would not have been amiss.

A wealth of material based on the authors' combined experiences is here available for all who wish to embrace the doctrine that management will prevail in the laboratory. So it might, but whether it will lead to better (and happier) laboratories remains to be seen. □

R.C. Holloway is a member of the scientific staff at the National Institute for Medical Research, Mill Hill, UK.

Biological NMR

Henry Rattle

Biological Applications of Magnetic Resonance. Edited by R.G. Shulman. Pp.595. (Academic: New York and London, 1979.) \$29.

BIOLOGICAL NMR has now reached the stage at which a burgeoning literature provides considerable problems both for those already in the field and for those hoping to enter it. The time is clearly ripe for good review articles, and if these articles are to be of maximum use they must be thoroughly up to date, critically selective in the literature reviewed and accessible to the reasonably knowledgeable but non-specialist reader. All of these requirements are admirably met in the volume under review, but in addition the editor has imposed a pleasingly practical direction on his contributors; for example, the lone article on EPR studies concludes with some brief notes on experimental design for biological materials, and that on ³¹P NMR in living tissue contains useful graphs of the pH-dependence of the chemical shifts of inorganic phosphate as well as design details of the special apparatus used in the experiments. An article on proton nuclear Overhauser effects begins with a very clear and minimally mathematical description of the nuclear Overhauser phenomenon, while one on the use of model compounds in the interpretation of the NMR spectra of haemoproteins starts with a clear table of the oxidation and spin states of iron and a brief qualitative introduction to the theory of hyperfine shifts in paramagnetic systems. Such concern for the non-specialist reader greatly enhances the usefulness of the book.

In all, the volume contains eleven articles averaging some 45-50 pages each. In addition to those already mentioned, they include reviews on hydrogen-bonded proton exchange and its effect on nucleic acid NMR spectra, on nucleic acid structural studies, drug-nucleic acid complexes, anti-body binding sites, multinuclear NMR of the structure of alkaline phosphatase, and the NMR of catalytic groups of serine proteases and of living *Escherichia* cells. All the authors are of high reputation and long experience in biological NMR, and the standard of articles remains high throughout. The volume is reasonably priced by modern standards and is highly recommended to anyone with more than a passing interest in the subject. □

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Long range order in solids

David Sherrington

Long Range Order in Solids. By R.M. White and R.H. Geballe. Pp.409. (Academic: New York and London, 1979.) \$39.50.

THE most obvious example of long range order in solids is the periodic arrangement of atoms which makes up the crystalline state. More generally, however, the concept pervades most of condensed matter physics at low enough temperature. Two other classic manifestations are ferromagnetism and superconductivity, the former relatively easily envisaged as long range order of spin orientation, the latter involving a more subtle order in momentum space. This book is an overview of the whole subject.

There is much common ground among different examples of long range order if one knows how to look. An appreciation of the universality is invaluable in using experience in one area to yield insight in another. One aspect of the book is a discussion of these common features, such as the concepts of order parameters, phase transitions and critical phenomena, the role of symmetry, excitations and so on. The main difference from earlier texts lies, however, in the wealth of experimental data used to illustrate and develop these ideas. Indeed, it is the authors' expressed belief that "a great deal can be learned by looking carefully at experimental data, and that the experience of doing so will develop judgement and intuition". Further believing that "clues to entirely new phenomena are often buried in existing data", they have presented a plethora of data, primarily, but not exclusively, taken from examples in magnetism, superconductivity and charge density wave ordering; within these fields it is difficult to find a topic which has not been touched upon. To complement this data compilation a substance index is included. Unfortunately, however, some potentially useful tables, while indexed under individual materials, are not indexed under the relevant generic names.

The stated level of the book is intermediate between a postgraduate course and a research seminar, and indeed this is about where it lies. Without a reasonably solid base in solid state physics and statistical mechanics the reader would find himself floundering quite often. Elementary many-body theory would be an asset, as also some appreciation of invariants, but one could manage without them. The other essential requirement is determination, because the cover (and often the style) is encyclopaedic but the book seems designed to be read from start to finish rather than

delved into. The student who completes the course will certainly have a broad knowledge at the end. He must, however, be prepared to dig into the research literature to pursue further any individual topic. He will be assisted by the references, which are very up-to-date (several 1978 references).

The book starts by introducing the general concepts of long range order, followed by phenomenological theories of phase transitions and excitations. It then discusses the mechanisms (real and hypothetical) leading to ordering in superconductors and magnets, and the experimental techniques available for probing them. Systematics of long range order in magnetism and superconductivity are followed by discussion of impurity effects and the coexistence of different types of order. In discussing impurity effects there is a divergence from the main subject to that of the formation of local moments in metals and the Kondo effect. Finally, there are chapters on domain structure and inhomogeneous phenomena and on long range order in amorphous and granular materials, these last being very active subjects of current research.

There is sometimes an uneasy mixture of

generality and detail, of different and sometimes inconsistent levels of assumed background knowledge, and of exact and approximate results. I consider it particularly unfortunate that in a book of this type and level approximate results are not always clearly labelled as such. I recommend caution to readers insufficiently experienced to distinguish them.

Overall, the authors have achieved their objective of demonstrating the generality of the concept of long range order, of outlining its language and tools and of showing the importance to its development of the analysis of experiment. This should be useful to a student who has a good basic foundation but lacks breadth. Whether the authors' hope that their organisation of theoretical concepts and experimental manifestations will "enable the reader to make associations which will lead to new ideas" remains to be seen. Let us hope that if it does the new discoverer will let them know. □

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A botanist's what's what

S.M. Walters

Elsevier's Dictionary of Botany 1. Plant Names. By P. Macura. Pp. 580. (Elsevier: Amsterdam and New York, 1979.) \$109.75; Dfl. 225.

THE publication of a new polyglot glossary of botanical words is an event of some importance for librarians and others who face the problems of information retrieval for pure and applied botany. This handsome new volume by Paul Macura of the University of Nevada gives the equivalent in English, French, German, Latin and Russian of more than 6,000 plant names, and a companion volume dealing with terms of a more general nature (excluding Latin) is in preparation.

The Preface claims a unique place in botanical literature for the book, apparently ignoring the existence of the *Botanical Dictionary: Russian-English-German-French-Latin* by N.N. Davidov published in Moscow in 1960. It is true that Macura's dictionary deals exclusively with plant names, for which there are more than three times as many entries as in Davidov; but the proof of the

pudding is in the eating, and in a random test of five English vernacular plant names, birch, bracken, honey fungus, lucerne and maize, Davidov scored four correct or tolerably correct answers, against only three for Macura. Both signally failed to recognise that the English (as opposed to American) word for the crop *Medicago sativa* is 'lucerne', not 'alfalfa'; and Macura seems to think that the English name for *Pteridium aquilinum* is 'adderspit', though he recognises 'bracken' for the western North American var. *pubescens*.

The decision to include in the primary alphabetical listing of English names a large number of "adjectival vernacular names" seems difficult to justify. It enormously expands the book with relatively little gain, and omissions and inclusions seem to be quite arbitrary. Indeed, a more ruthless selection of original material could have produced further economies by eliminating, for example, 'goldmoss' which immediately precedes, and refers directly to, the next entry (No. 2553). The verdict must be that the dictionary is useful, but that it could have been more carefully checked; it could also have been much more compact and therefore cheaper. □

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OBITUARY

R. B. Woodward, 1917–1979

THE sudden death of Robert Burns Woodward of Harvard University on 8 July 1979 at the age of 62, marked the passing of one of the most important organic chemists of this century.

In offering the following account of some aspects of Woodward's achievements the main scientific emphasis will be on his contribution to chemical syntheses. I will leave to others a discussion of the celebrated Woodward-Hoffmann rules. It would, however, be wrong to undertake this exegesis before giving some idea of Woodward's impact on those — and they were hundreds — who came in contact with him.

Woodward's impact on his associates came first from an awesome command of the whole of organic chemistry, ranging from the incredibly complex mass of interrelations and transformations which chemists had produced while unraveling the molecular architecture of complex natural products, such as quinine, morphine or strychnine; to the ever more rapidly accumulating data on the course of chemical reactions and their theoretical basis; extending finally to the multitude of transformations, not excluding the most obscure or esoteric, which might be put to use some day in the rational construction of a complex structure.

Woodward's great strength was his conviction, which he conveyed to his associates, that chemical problems should be placed in a rigorously rational framework; leaving no room for fuzziness, for half understood data, for any but the most mercilessly logical deduction, whether dealing with the structure determination of an unknown natural product, or of an intermediate in a synthetic sequence. Woodward's intense commitment to organic chemistry was allied to the conviction that there must be underlying order beneath what often seemed a chaotic mass of unrelated observations. This approach showed fruit very early (1941) in the well-known "Woodward rules" which provided a generally useful correlation of important structural features of α , β -unsaturated ketones with their ultraviolet absorption maxima.

This early recognition of the importance of the informed application of physical methods to structural problems led Woodward to give a major role to the emerging tool of infrared spectroscopy in his rigorous deduction of the β -lactam structure of penicillin (1944–45). This, and the later conclusion that the vast amount of the existing strychnine chemistry demanded revision of the accepted structure to a different one, now known to be correct, are early examples of



Woodward's mastery of this highly intellectual form of detective work. His skill in presenting his conclusions in a dramatic and lucid style makes his accounts of the elucidation of the structures of strychnine and, *inter alia*, of the tetracyclines or tetrodotoxin fascinating and highly rewarding reading. This is true even today, in spite of the fact that such problems have largely been taken away from chemistry by advances in X-ray crystallography.

The faith in an underlying rational structure showed itself also in Woodward's brilliant use of the "curved arrows" which Robinson's genius had earlier introduced into organic chemistry, to indicate the breaking and forming of bonds, and which have become an essential conceptual tool in visualizing the path from reactants to products. It was Woodward, more than anyone else, not excluding Robinson himself, who showed that Robinson's curved arrows could be submitted to rigorous logical constraints, and that, when they were thus used, they would greatly assist, not only in systematizing observed reactions, but also in predicting which reaction might be possible, and which unlikely. An early example (1948; 1950) of Woodward's command of this tool can be found in his unraveling of some baffling molecular transformations in the Santonin series.

There is one area of chemistry which is most uniquely the domain of organic chemistry, and that is molecular architecture, or synthesis. As chemists entered the twentieth century, the very small number of reactions which might be useful in reaching the complex targets represented by natural products such as cocaine, quinine, morphine or the steroids, coupled with a general lack of knowledge

of ways to control stereochemistry, the three dimensional arrangement of atoms in space, made the challenge of total synthesis of most natural products as formidable as it was exciting. Nevertheless, some beginnings were made: Willstätter synthesized tropinone, the parent of cocaine, around 1900. This was a work of genius, given the state of development of chemical synthesis at that time, but the labored nature of the synthesis also served to emphasize the enormous problems to be faced by would-be molecular architects.

It must have been as important psychologically as it was chemically startling, therefore, when Robert Robinson's famous construction of tropinone burst upon the chemical world in 1917. Willstätter then succeeded in transforming tropinone into cocaine in 1923: the possibility of other complex total syntheses could now be contemplated. Some progress on methodology came from "partial synthesis," the transformation of one natural product into another (e.g. ascorbic acid from hexoses by Haworth, and by Reichstein, in 1933); the forbidding, though achiral, haemin was put together by H. Fischer in 1928 by a combination of steps which must have convinced any would-be practitioner that witchcraft must play a large part in a successful synthesis. Probably the greatest achievements in the decade preceding the second World War were the synthesis of dihydroquinine by Paul Rabe in 1931, and that of equilenin by Bachmann in 1939.

It is on that stage that R. B. Woodward made his dramatic entrance with the announcement of the synthesis of quinine (with W. E. Doering) in 1944. The synthesis commanded worldwide attention because of the burst of publicity (front page of the *New York Times*: "Second Answer to Japan") (the first, being, supposedly, synthetic rubber) which propelled Woodward, then twenty-seven, to a limelight which he did not altogether dislike. Dramatic as it may have seemed to the press, Woodward's quinine synthesis closely followed the scheme laid out by Rabe in his hydroquinine synthesis. It shares with that early synthesis an almost total lack of stereochemical control. And yet, it is of great importance because, in connection with the construction of a piperidine derivative, it introduced what I believe to be one of Woodward's most important contributions to the strategy of complex synthesis: the formation, manipulation and eventual cleavage of rings of carbon atoms as a method of construction of acyclic elements. This approach led to a considerable simplification of the route to the target structure, since it allowed carrying

functional groups in a latent, less reactive form: in the piperidine precursor of quinine, an eventual ϵ -aminoheptanoic acid system is constructed by the cleavage of a 2-methylcyclohexanone, a chemically less reactive, and thus more easily controllable system.

The modern era of concern for stereochemistry started in the period 1950-1955, a period which saw the development of stereospecific, or highly stereoselective, syntheses of morphine, cantharidin, cortisone, cedrol and strychnine. These syntheses were all carried out without taking advantage of the enormous help, just becoming available, of Barton's principles of conformational analysis, but they are important because they represent the first successful efforts to take stereochemistry explicitly into account during the process of synthesis planning. This may seem an obvious prerequisite to a successful synthesis, but one need only read the highly imaginative, though largely inconclusive, series of some sixty papers on steroid "synthesis" by Robinson and his collaborators to see the staggering efforts which could be made to reach synthetic targets (steroids in this case), without any concern for the need to control the relative arrangement of the relevant atoms in space. When one considers that without such control, the connectivity implied by the two-dimensional structure of cholesterol represents 256 different substances, only one of which is cholesterol, it is no wonder that these efforts, brilliant as they sometimes were, remained largely abortive.

Although Woodward's 1951 steroid synthesis was only partially stereoselective in contrast to the cortisone synthesis (by L. H. Sarett) referred to above, it was nevertheless highly influential because it against used the structural simplification made possible by a temporary ring: a cyclohexene ring serves as the source of the steroid D-ring via its cleavage and cyclization to a more functional cyclopentene aldehyde. One also sees here an illustration of another important principle of many Woodward syntheses: the carbon framework is constructed as rapidly as possible, leaving some function (e.g. double bond) which will allow eventual structural adjustments.

It is with the synthesis of strychnine, in 1954, that Woodward began a series of classically elegant syntheses which have not yet been surpassed. In addition to strychnine, they are those of reserpine (1956), of chlorophyll (1960) and of the "western half" of vitamin B₁₂ (1972). In the strychnine construction, the ubiquitous ring element, placed in the α -position of the indole ring of tryptamine, is a dimethoxybenzene ring which is here a latent 1,4 butadienedicarboxylic (muconic) ester system, in a much less reactive form. Before that transformation, however, that same ring is brilliantly used to ensure that an intramolecular cyclization via an

iminium salt occurs at the β -position of an indole ring. The synthetic use of a dimethoxybenzene ring as a precursor of a muconic acid illustrates an aspect of the Woodward mastery which has been referred to previously: the cleavage of an ortho-dimethoxybenzene to a muconic acid had been stored in Woodward's memory since he had noticed its use by Speyer in the degradation of codeine. He was able to recall it just when it would be most dramatically useful.

In the synthesis of strychnine, details of oxidation states were consciously held in the background because of the great structural simplification thus allowed in the construction of the framework. This approach is further refined in the next great synthesis, that of reserpine, in which full and impressive use is made, for the first time, of Barton's conformational principles. In that synthesis, we have, once again, the use of a cyclohexene ring as a surrogate for an eventual aldehyde-ester. We also see here the dramatic simplification produced by the use of a double bond as the precursor of the monomethyl ester of a 1,2-glycol. There were, *a priori*, four possible such systems which might result from the cyclohexene double bond. Woodward had acquired, by that time, extraordinary confidence in his ability to find some way to make molecules dance to his tune: he felt that something might well happen during the synthesis, which would solve this potentially vexing problem. He was not disappointed. Only someone with Woodward's ability to deduce structures from a combination of limited spectral data and mechanistic insight could possibly have capitalised on the remarkable chemical events which provided a solution to the problem. Seldom has it been more true that "chance favors the prepared mind."

Chlorophyll and the "western half" of vitamin B₁₂ will end this broad sketch. In the synthesis of the former, it is still notable that a problem in producing the required regiochemistry was solved by selecting an aminoethyl group as precursor of an eventually required vinyl substituent, and using it in temporary ring formation with a pyrrole aldehyde. Again, the very difficult problem of introducing the two so-called "extra" hydrogens specifically in one of four pyrrole rings was solved, in a unique manner, by a sequence involving cyclization followed by cleavage.

Finally, when we come to the B₁₂ synthesis of 1972, we are treated to a pyrotechnic display of appearing and disappearing rings which are used here to achieve control of almost all the asymmetric centers in the molecule. A propionic acid chain arises from the remains of an anisole ring, and another one from a temporarily constructed cyclohexene. A δ -ketoacid originates from the cleavage of a cyclopentene; an amino acid system is born from a cyclopentanone, as yet further rings make

their tightly orchestrated entrances and exits from the B₁₂ stage: a grand final tribute to the power of this approach to synthesis.

I referred at the very beginning to the great impact that Woodward had on all who associated with him. This was nowhere more obvious than in the celebrated "problem seminars" in which Woodward was always willing to take on all those who cared to match wits and deductive skills with him. It was not just the graduate students and postdoctoral associates who benefitted from contact with Woodward's logical approach to all chemical problems: an impressive number of papers by celebrated chemists all over the world (Arigoni, Bartlett, Barton, Bloch, Djerassi, Eschenmoser, Gates, Inhoffen, Jeger, Klyne, Prelog, Wilkinson, Winstein, Witkop. . .) have Woodward as a coauthor, obvious testimony to the insights that were gained by discussions with him.

Woodward would not have been human had he not enjoyed the attention he commanded. He relished keeping audiences enthralled for hours with a lecture in which he obviously savoured highlighting the already brilliant work he was describing by using meticulously drawn formulas, dramatically enhanced by the controlled use of colored chalk, further to emphasize the image of one in total command of his environment. He was not above playing along with the sensationalism of the media: "That is what the public wants of its heroes"; and he had no doubt that he had earned the right to special recognition at every level. One anecdote, perhaps, sums up the view he had of his rightful place in the world: A new guard at Harvard had just told Woodward that his (blue) car could not be left where he had placed it. "Why is that?" said Woodward. "Your name is not on the list," said the guard. "It isn't?" said Woodward, turning back toward the guard without stopping, "well . . . put it there!" One thing is certain, Woodward's place in chemical history is permanently reserved.

Gilbert Stork

G.S. Forbes

GEORGE SHANNON FORBES, Emeritus Professor of Chemistry at Harvard University, died on 24 June 1979 in his home in Cambridge, Massachusetts.

Born on 17 March 1882 in Boston, Massachusetts, Forbes' interest in science was awakened at the age of eight, by a lecture on pendulums given by his father, George Fairfield Forbes, who was a pioneering teacher of experimental science at the prestigious Roxbury Latin School. But when young Forbes graduated from that same school he aspired to a career as a professor of classics. During his sophomore year at Harvard College, he reports, in a course on qualitative analysis the

challenge of numerous "diabolical mixtures . . . aroused my combative instincts, and provided real excitement . . . Gradually the classics were supplanted by a new enthusiasm. Then followed a truly decisive event. Professor Charles R. Sanger told me that next year's class would be larger than usual. Would I serve as assistant without stipend? Of course I said 'Yes,' and so, not long after my eighteenth birthday, I became a duly appointed officer of instruction in Harvard University, continuing on through senior year . . . Ten years later, this adventure had a direct influence on my fortunes. When Professor Sanger's health became impaired, I was asked to collaborate with him. Upon his death, I took charge of qualitative analysis, and thus my feet became planted upon the academic ladder."

By that time Forbes had long since passed from undergraduate to graduate work at Harvard. Under the direction of T.W. Richards, later to become America's first Nobel laureate in the sciences, Forbes completed in 1905 a doctoral thesis on the electrochemistry of amalgams. He then went on with Richards to a revision of the atomic weights of nitrogen and silver. The keystone of this endeavour was a meticulous redetermination of the exact weight of pure silver nitrate yielded by one gram of pure silver, and the result thus obtained still stands unchallenged (good to 0.002%). But this notable first was also Forbes' last investigation of atomic weights. Most of his nearly 100 research publications deal with solution physical chemistry, especially electrochemistry, and with photochemistry, in which field he was an American pioneer.

Forbes' life and career were wholly centered on Boston and Cambridge Massachusetts. To be sure, he spent 1906-07 at the universities of Leipzig and Berlin, followed by two years as associate in chemistry at Bryn Mawr College. But in 1909 he returned as instructor in physical chemistry to Harvard University, and here he stayed to become Professor in 1926, Chairman of the Chemistry Department in the difficult years 1944-47, and Emeritus Professor in 1948. Even then Forbes' academic career continued for 8 years more Northeastern University and, still persevering as a consultant to chemical industry, inuing as a consultant to chemical industry, he continued laboratory work with his own hands until the age of 90. Triumphant over the hazards besetting the experimental chemist, he was at 97 the oldest living graduate of Roxbury Latin. He outlived his cherished wife of 60 years, *née* Marie Louise Hersey, but is survived by a son, a daughter, and four grandchildren.

Born into a family where father, mother (*née* Elizabeth Shannon), aunts, and uncles were all teachers, Forbes came naturally to that vocation. His teaching career spanned 60 years and some 9000 students. A highly-organized lecturer who wrote everything out in a well-ordered display on the black-

board, Forbes was also a willing spontaneous commentator on the illuminating aberrations of the innumerable lecture experiments he dextrously produced. He was in 1951 the deservedly first recipient of the James Flack Norris Award for outstanding achievement in the teaching of chemistry.

Forbes was a New Englander through and through: sturdy, spare, independent, reserved, and patient. But beneath the somewhat austere exterior lay abundant dry wit, and a human warmth that engaged many friends. An attractively multi-dimensional person, Forbes was a photographer of professional competence, an energetic hiker and climber (long the faculty adviser of the Harvard Mountaineering Club), and an avid musician who lent his (double bass) voice to many local choruses. His high sense of responsibility found expression in a lifetime of devoted service to his science, his Department, his University, and his Church. His was a New England life of little showiness but admirable substance.

Leonard K. Nash

Sir Frank Fraser Darling

THE death of Sir Frank Fraser Darling on 22 October 1979 at the age of 76 will be greatly regretted by naturalists and wildlife conservationists in many parts of the world. Although he only came to wide public notice in Britain when he gave the 1969 Reith Lectures, he had made outstanding contributions to the understanding of wild animals and of the countryside over a period of some 50 years, and his counsels were much sought after in America and elsewhere in the 1950s and '60s, when his own countrymen paid less attention to his opinions.

He started work in 1924 as an agriculturalist. After three years on the staff of the Buckinghamshire County Council he went as a research student to the Institute of Animal Genetics in the University of Edinburgh. He was appointed Chief Officer of the Imperial Bureau of Animal Genetics in Edinburgh in 1930, under the late Professor F.A.E. Crew, and he remained in that post until 1933. He then gave up this urban, academic life, and began his work, often under conditions of considerable austerity, in the highlands and islands of Scotland. He held Carnegie and Rockefeller research fellowships, and was Director of the West Highland Survey from 1944-50, but this was a precarious existence with little of the security scientific workers expect today. However, he was able to spend his time in the field really getting to understand the conditions of his harsh environment.

During this period he wrote many books such as *A Herd of Red Deer* and *A Naturalist on Rona*, vividly describing his

observations and his experience. His *Bird Flocks and the Breeding Cycle* initiated a new field of research. These books had a considerable appeal to other naturalists and to discerning scientists, but were dismissed as too "popular" by the educational establishment, and so did not receive the recognition his admirers felt they deserved. He was bitterly disappointed at the reception given by "the authorities" to his West Highland Survey. His apparently eccentric behaviour and unconventional way of life (something which would be thought normal today) also prevented him from being properly appreciated as an observer and an ecologist in academic circles. At a time when laboratory studies of animal cells or isolated organs were considered more scientific than those involving whole animals in the wild, and when even meticulous observation was considered inferior to experimentation, it is not surprising that he felt he was not properly appreciated.

He did return to academic life for a few years when he was appointed Senior Lecturer in Ecology and Conservation at Edinburgh University in 1953 at the age of nearly 50. He was also invited by the Nature Conservancy in Britain, then a young and developing organisation, to advise them in their studies on Red Deer. This work, and his earlier observations, enabled the Conservancy to introduce a proper management policy particularly to the island of Rhum, where within the National Nature Reserve populations of deer were maintained at optimum levels by a careful culling policy. However, Fraser Darling increasingly found that his views were being taken more seriously in the United States of America, and in 1959 he accepted an invitation from Dr Fairfield Osborn to become Vice President of the Conservation Foundation in Washington DC. His studies of the caribou in Alaska with Dr Starker Leopold, and of the larger mammals in Northern Rhodesia and other African territories did much to consolidate his international reputation.

While in the United States he was impressed with the work done there in setting up National Parks, and he was critical of other countries, including his own, for their slowness in following the American example. However, he praised the British Nature Conservancy for their work, with limited resources, and particularly for establishing several valuable reserves in Scotland.

As already indicated, it was not until 1969 that Britain recognised his contribution to conservation. 1970 had been designated European Conservation Year, and plans to that end were being made by a committee under the patronage of HRH Prince Philip. It was suggested that the 1969 Reith Lectures of the British Broadcasting Corporation should be devoted to some suitable ecological topic, and that they should act as a curtain raiser.

Fraser Darling was an inspired choice as the speaker. He took as his title *Wilderness and Plenty*. The lectures came at the right time, and the lecturer made the most of his opportunity. The public, as distinct from the scientific community, had just learned to use the word "ecology", and to be worried about the future of the environment. The impact of the lectures was considerable, and Fraser Darling was accepted, particularly by students, as a prophet. His status was also radically changed and, somewhat to his surprise, he found himself very much a part of the Establishment. The Queen honoured him with a knighthood, and he was in demand to join the most prestigious Quangos.

Thus from 1970 to 1973 he served on the Nature Conservancy, and in 1970 he was one of the first selected for the Royal Commission on Environmental Pollution. However, he was not seduced by his acceptance by the authorities, and still continued to speak his mind on environmental problems. He was in great demand as a speaker and a writer, but unfortunately ill health prevented him, particularly in recent years, from playing an active part in public life.

It is difficult to assess Fraser Darling's contribution to science at this time. His books show his deep but unsentimental feeling for the countryside and for the animals which inhabit it. He was able to communicate to the public (as in the Reith Lectures), and although this very ability aroused disapproval in some (? envious) academics, it enabled him to make a unique contribution to conservation. However, many of his colleagues, even those who admired him most, did not consider him to be an outstanding ecologist in the academic tradition, and it is not difficult to pick holes in some of his work. Nevertheless he was a leading figure who followed in the British tradition of the study of scientific natural history, and we can take comfort from the fact that at least for the last years of his life this was realised by his countrymen.

Kenneth Mellanby

C.S. Hallpike

DR CHARLES SKINNER HALLPIKE, CBE, FRS, FRCS, FRCP, who died on 26 September 1979, aged 79, was a neuro-otologist of international renown. He was the pioneer in that field and is respected as such in every country of the world.

Although an experienced physiologist and pathologist, Dr Hallpike's special interest was in the relationship of experimental observation to clinical problems. His ability for meticulous work and great clarity of thought enabled him to marshal facts and produce scientific papers in stylish and fluent prose. He is best remembered for his original description of the pathology of Ménière's disease and for the development of a caloric test technique which has, over the years, withstood the

test of time and is now universally accepted.

Dr Hallpike was educated at St Paul's School and graduated in medicine at Guy's Hospital, London in 1926 where he was Entrance Scholar in Arts and Beaney Prizeman in Pathology. His interest in the ear began when he was House Surgeon to the aural departments of Guy's Hospital and Cheltenham General Hospital from 1924 to 1927. Thereafter, as Bernard Baron Research Fellow at the Ferens Institute of Otolaryngology, Middlesex Hospital, the publication of a number of papers concerned with the electrophysiology of hearing resulted in his being awarded the Duveen Travelling Studentship, University of London, 1930 and the Rockefeller Travelling Fellowship 1931, when he studied with Witmarck in Germany and in the University of Philadelphia, USA. His high standard of scholarship received further recognition in the awards of the Foulerton Gift Research Fellowship, Royal Society (1937-40) and Gamble Prize in 1934, William J. Muckle Fellowship, University of London, 1941 and Dalby Prize, 1943.

In 1942 he joined the scientific staff of the Medical Research Council at the National Hospital, Queen Square, London where his power of organisation and administration resulted in his appointment as Aural Physician and Director of a newly established Otolaryngological Research Unit at the hospital. Here, with his awareness of the close liaison which should exist between laboratory and clinic in the solution of problems throughout the field of otology, his work became directed towards establishing this link to the great advantage of both. In association with experts in numerous allied disciplines including physics, physiology, biochemistry, histology and clinical investigation, a series of classic publications issued from his unit emphasising the importance of the adoption of quantitative methods in both laboratory and clinical studies of the ear.

The practical outcome of this work included the Medresco hearing aid, the peep-show technique for measuring deafness in young children, recognition of the diagnostic significance of loudness recruitment in sensori-neural deafness and the definition of a number of clinical and pathological entities hitherto grouped together as 'aural vertigo.' An improved technique for temporal bone microtomy was developed, a rotating chair, a head lamp for aural surgery and an ear microscope. Further studies contributed to knowledge of the heredity of labyrinthine disease and the biochemistry of the labyrinthine fluids. At a later stage the narrow-band masking technique for bone conduction audiometry was evolved and an electro-nystagmographic technique, the diagnostic significance of which is still increasing.

Dr Hallpike's unit gained international recognition in the awards he received of the

Gamble Prize for the second time and the Hughlings' Jackson Lectureship and Medal, Royal Society of Medicine 1947; Bárány Medal, University of Uppsala 1958; Guyot Medal, University of Groningen 1959 and the Dalby Prize (awarded jointly) in 1958. In 1956 he became a Fellow of the Royal Society and in 1958 he was honoured with a CBE.

Upon his retirement from the staff of the Medical Research Council, Dr Hallpike was appointed Honorary Aural Physician and Director of Research at the Ferens' Institute of Otolaryngology, Middlesex Hospital from 1965 to 1968. He was a member of numerous learned societies including the Collegium Otorhinolaryngologicum Amicitiae Sacrum whose Shambaugh Prize was awarded to him in 1955. He was an Honorary Fellow of the Royal Academy of Medicine of Ireland, a Fellow of the Royal Society of Medicine, being Honorary Secretary 1938, Editorial Representative 1946 to 1952 and Honorary Member of the Section of Otology 1970. He was joint Founder of the Bárány Society in 1960, a society which now has world-wide membership and is unique as being solely directed to the study of vestibular problems. Between 1938 and 1965 he was a member of the Flying Personnel Research Committee where his almost unique knowledge of the ear's functioning in health and disease played a large part in overcoming aural problems important in aviation.

Dr Hallpike had the great merit of having inspired many collaborators to engage in scientific work and his wisdom and shrewd judgement will be remembered the world over. He was the acknowledged master of his subject, albeit a hard one, a perfectionist, utterly single-minded in all he undertook. He was quick to recognise and appreciate good work, intolerant of fools but unfailing in giving due credit to his co-workers. The fact that the majority of his unit staff remained with him over the years, a number from its inception until his retirement, is testimony in itself to his integrity. He had enormous courage in the face of an orthopaedic disability resulting from a childhood injury which precluded many outside activities. He played billiards in his younger days and enjoyed rifle practice at Bisley for which he gained a number of awards. He played the piano and violin and was fond of classical music. He enjoyed gardening after his retirement and won prizes locally for his roses. He was a devoted family man and is survived by his wife, Barbara, and two sons.

His death is a great loss to British otology. The establishment of the Medical Research Council Hearing and Balance Unit at the National Hospital, Queen Square, under the directorship of Dr J.D. Hood, with other members of his old staff, remains a living tribute to his pioneer work.

M.R. Dix

nature

3 April 1980

Chemical arms: a sea of troubles

RECENTLY, the worldwide press has reported on how rumours of Soviet chemical warfare in Afghanistan have led to increased support in the US for the resumption of manufacture of chemical weapons. Europe should take note of these reports, for if US chemical weapons production does resume it will almost certainly have been endorsed beforehand by at least one European government. Prior experience suggests that such endorsement will not receive prior parliamentary debate.

For several years European members of NATO have been under pressure from the United States to reconsider their positions on chemical weapons. Existing NATO policy is that chemical weapons are to be used only for retaliation in kind within the prevailing 'flexible response' defence strategy. US officials believe that this policy requires NATO to integrate a retaliatory chemical capability much more closely into its military planning and posture than it has so far done. France and the US are alone within the alliance in possessing significant stocks of poison gas, and, although certain other member-states, such as Britain, keep themselves informed about how to use chemical weapons, the remainder do not. West Germany, in particular, has long committed itself against acquiring its own, and has declared that it will not seek access to anyone else's.

The prevalent European view has been that the threat of escalation (including nuclear weapons release) implicit in the flexible response strategy, coupled with the antichemical protection of NATO forces, provides sufficient safeguard against Soviet resort to chemical warfare. Antichemical protection is currently being upgraded, and is capable of negating the mass-destructiveness of chemical weapons on the battlefield, albeit at some cost to fighting efficiency. French and American chemical weapons are seen as a useful extra precaution to the extent that they can act as an additional deterrent — a belief which may or may not be sound.

Suggestions that the NATO chemical retaliatory capability should be expanded have been opposed on several grounds. First, such an expansion could be interpreted as a sign of diminished resolve to use nuclear weapons. Increased deterrence of chemical warfare would then have been bought at cost of reduced overall deterrence. Secondly, there would be greater returns for European security from investment in improved conventional capabilities. In addition, a new chemical armament drive would compromise the current negotiations on chemical disarmament, which in theory offer the best safeguard against chemical attack.

While many Americans appreciate such arguments, some maintain that the present state of nuclear parity now thought to exist between the US and the USSR, together with the apparent Soviet interest in chemical weapons, strengthens the requirement for a special chemical-warfare deterrent. For reasons that are not entirely clear, they believe that their existing special deterrent — in the form of the one to two weeks' supply of US chemical munitions now on hand in West Germany, replenishable from much greater American stockpiles — is insufficient. Hence the plans for a new chemical weapons factory with a projected expenditure, so it is said in the American press, of \$1,300 millions, on an output of nerve gas artillery shells, bombs and warheads.

Such a programme can make sense only if Europeans undertake, at the very least, to accept the new weapons on their soil. This is the endorsement which potential host countries will be asked, once again, to give.

There are few public signs of the way European opinion may go on poison gas. The Afghan allegations, however unsubstantiated, could be influential, succeeding as they do a whole series of others that the USSR will use chemical and even biological warfare whenever it is militarily expedient, regardless of treaty

constraints. Reports have alleged Soviet backing for Vietnamese chemical warfare in Laos and Kampuchea, expansion of Soviet chemical-weapons stocks, and Soviet violation of the 1972 Biological Weapons Disarmament Convention. Yet, as a recently published review of these different charges shows in some detail*, no solid evidence has been disclosed for any of them.

Should the allegations be true, the implications for the West would be serious. The problem is that uncertainty about them extends to government — a symptom of the generally poor state of western chemical warfare intelligence. Because there is little hard information, these reports are in fact rooted in worst-case assumptions. Capabilities are estimated by equating them with the requirements for chemical weapons set by Soviet military doctrine on chemical warfare which, though not known with any precision, is assumed to teach maximum exploitation of all battlefield target effects available from toxic chemicals. The stockpile tonnage estimates quoted recently in the US press (*Nature* 13 March, 1980) are no exception. Their belittling of American capabilities is also commonplace, compounded in this instance by a confusion of chemical weapons with poison gas: it is the latter, not the former, of which the Americans have 42,000 tons — a supply sufficient to fill up to half a million tons of chemical munitions.

Absurdly, such estimates of Soviet stocks are frequently quoted by military analysts and journalists as evidence of the intentions from which, in fact, they were deduced. Because the Soviets have an enormous supply of chemical weapons, such comment goes, they must be planning for protracted chemical warfare of their own initiation. Yet such hard quantitative information as there is, drawn mostly from sightings of Soviet depots in forward areas believed to contain chemical weapons, is equally consistent with a retaliation-only posture, similar to NATO's.

In the historical record of US and Soviet chemical warfare preparedness since World War II, there is clear evidence that the programmes of one side have driven, at least in part, those of the other. The US stopped adding to its stocks of chemical weapons in 1969; the USSR, a year or two later. It would be highly dangerous if a cycle in the opposite direction were now to be set in motion again. The chemical arms limitation talks in Geneva, which are proceeding in both a US-Soviet working group and within the 40-nation UN Committee on Disarmament, provide what is probably the only available channel of communication for resolving such uncertainties. The first priority for the West in its policy-making on chemical warfare must therefore be to keep this channel open.

Over the years the negotiations have advanced to the point where they are now turning on intricate questions of the controls that must be placed on national chemical industries, and on particular military facilities, in the interests of adequate treaty verification. These raise issues of such delicacy that negotiation can proceed only if there is mutual confidence.

If the Soviets are innocent of the chemical and biological warfare allegations against them, a new western chemical armament drive cannot fail to deepen mistrust and suspicion. Although a go-ahead for the US binary nerve-gas plans could strengthen the position from which the West is negotiating, it could at the same time destroy the basis of negotiation. While Europeans may not be able to do very much to promote chemical arms control, they may soon be in a position to kill it. □

**Chemical and Biological Warfare: Analysis of Recent Reports concerning the Soviet Union and Vietnam, March 1980, pp42. Available as ADIU Occasional Paper no 1, from the Armament & Disarmament Information Unit, University of Sussex, Brighton BN1 9RF, UK price £1.50 plus postage.*

United States

Inventorship dispute stalls DNA patent application

Is scientific history being rewritten to make research results more patentable? David Dickson reports

A PATENT application covering some of the basic techniques of genetic engineering is being held up by the US Patent Office in a dispute over whether the applicants can legally claim to be the sole inventors. The patent request has been filed jointly by Stanford University and the University of California, San Francisco, (UCSF) on behalf of Dr Stanley Cohen and Dr Herbert Boyer. It covers techniques developed at the two institutions in the early 1970s, which demonstrated for the first time the feasibility of replicating biologically-functional foreign genes introduced into a living organism.

Central to the patent application, first filed in 1974, is a paper published in the *Proceedings of the National Academy of Sciences* (70, 3240, 1973) in the preceding year, which describes the successful construction and replication of a plasmid capable of transferring antibiotic resistance into *Escherichia coli*.

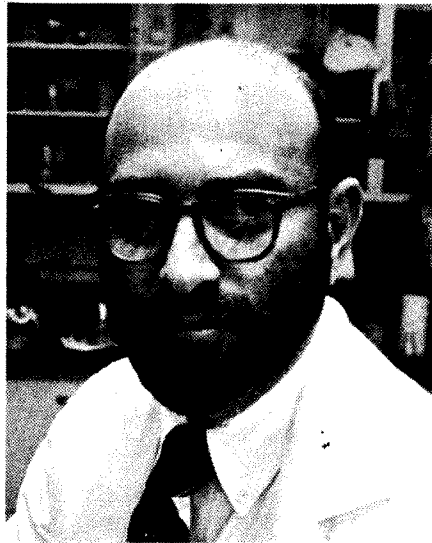
In addition to Cohen and Boyer, the paper names two other authors Dr Annie Chang of Stanford and Dr Robert Helling, Associate Professor of Botany at the University of Michigan. According to the rules of the Patent Office, joint authorship means the two are considered co-inventors of the processes described.

Stanford and UCSF argue that although the research described in the paper and the patent application was built on techniques developed elsewhere, it was Cohen and Boyer who provided the creative input necessary to demonstrate their potential. They did this by conceiving and demonstrating how the techniques could be combined to create functional recombinant plasmids.

But Helling, who is not named in the patent application, disputes that his role was marginal. He has refused to sign a disclaimer, required by the Patent Office before it approves the application, agreeing that he was not an inventor of the processes described.

"I felt that we were all equal in this, and do not want to sign a letter saying that I was just another laboratory worker", he told *Nature* last week. "I was part and parcel of the whole thing; I don't feel that I should sign something that I do not believe is true."

Helling is not the only scientist who has refused to sign (although his refusal has been the most disruptive). Dr John Morrow, of Johns Hopkins University, the first author on a 1974 *PNAS* paper cited by



Cohen: forgoing royalties

Cohen and Boyer (to show how a bacterial plasmid could be used to reproduce *Xenopus* DNA), complains in particular about the secrecy which has covered the application. "I am not prepared to sign a disclaimer to a patent application that I have not seen," he says.

And there may be further disputes in store. In addition to the process patent, Stanford and UCSF are also asking protection for the plasmid developed, arguing that it was the development of this particular pSC101 plasmid that made the success of the techniques possible.

Meanwhile, patents are being granted on the processes used. Dr Roy Curtiss, of the University of Alabama, has recently been granted a patent for the techniques used to create the disabled χ 1776 strain of *E. coli* (for which both process and product patents have already been issued in the UK).

The issue has raised at least two separate arguments. The first is on the morality of granting private licensing rights to the results of research carried out on public funds. As this is currently accepted practice, most scientists feel that Stanford and UCSF are justified in seeking to benefit from research which the two institutions pioneered — particularly since the two universities stress that the patents would remain freely available to anyone who wanted to use the techniques for research purposes. And both Cohen and Boyer have agreed that royalty proceeds should go to

support research at the universities, and that neither will benefit directly.

A further difficulty arises from the clash between scientific conventions of co-authorship, and the legal implications of these conventions when it comes to claims of inventorship. The latter is a legal definition which courts have ruled is enshrined in the Constitution, but is seldom considered by scientists in determining whose name should go on a research paper. Patents can be granted for techniques whose details have been published less than a year previously, but where such prior publication has taken place, all those listed as co-authors are treated as *prima facie* co-inventors.

In the Stanford/UCSF case, all agree that the development of recombinant DNA methodologies relied heavily on discoveries made at several institutions, for example on work on restriction enzymes at Johns Hopkins University and by Paul Berg's group at Stanford, or on the functioning of ligases at the National Institutes of Health.

As Cohen puts it: "Scientific advances such as the one we have been involved in are in fact the result of multiple discoveries carried out by many individuals over a long period of time". The difficulty lies in evaluating the significance of any particular contribution.

Patent attorneys for the two universities argue that Boyer and Cohen provided the "major inspiration and direction" for the experiments described in the *PNAS* paper, and they have told Helling that, unless he signs the disclaimer — or they can convince the patent examiner by other means that he has no legal claim as an inventor — then they will seek a court order instructing the issuing of a patent, and 'deposing' him as an inventor. But a full-scale legal dispute is not a prospect that anyone fancies.

Legally-defined constraints imposed by commercial considerations are proving a problem for scientists concerned to retain the integrity of the research process.

"It is difficult to demand high standards of your students when you see some of the things that are going on", says Dr Mark Ptashne of Harvard University, who claims that colleagues are beginning to write papers and cite references. "In such a way that their patent applications are valid".

Dr Zsolt Harsanyi, who is currently heading a major study of the implications of recombinant DNA technology for the office of Technology Assessment, confirms that "in the past couple of months" scientists have begun to talk about the potential for rewriting scientific history as a "serious problem".

Some are now using this as an argument against permitting the patenting of research results at all. Others suggest that fields such as electronics and chemistry have faced such conflicts for years, and have learned to live with the consequences.

In the case of molecular biology, the learning process does not look like being a comfortable one. □

Third World science fund: promises unfulfilled

THIRTY-five countries last week pledged a total of \$36 million to a new fund being set up by the United Nations Development Programme to assist developing countries build up their scientific and technological capabilities.

The fund was agreed as an interim measure at last summer's United Nations Conference on Science and Technology for Development, held in Vienna. UNDP officials estimate that extra commitments already made increase the amount pledged to \$45.7 million; a number of other countries are expected to make additional contributions over the next few months. However, the figure reached is far short of the \$250 million which delegates to the Vienna conference, agreed should be a minimum target for the fund (contributions to which would be voluntary).

The developing countries had proposed a more ambitious financing system at Vienna, based on automatic contributions and aimed at targets of \$2 billion by 1985 and \$4 billion by 1990. But the developed countries put forward the proposal for the interim fund as an alternative. This fund is initially designed to operate for the two years 1980-81: during this period, a group of specialists will meet to decide whether the more ambitious proposal is a feasible proposition, and if so how it should be carried out.

Meanwhile plans are already being drawn up by the UNDP for allocating the money contributed to the fund. It is meant to support the implementation of recommendations agreed at Vienna, such as promoting scientific cooperation between developing countries on a subregional and regional level, and seeking to strengthen their "endogenous scientific and technological capacities".

Last week's pledging conference in New York was attended by delegates from 78 countries. Almost all said that they supported the goals of the fund, although many indicated that their governments had not yet had time to agree on the exact size of contribution to make, while others claimed that short-term economic problems made it impossible to make a contribution now.

Several contributions were received from developing countries themselves. These admitted that the size of the payments they were able to make was small compared to the overall target, but emphasized that the contributions were intended primarily as a token of support for the fund. Thus Samoa pledged \$1000, Tanzania pledged 30,000 Tanzanian shillings (equivalent to \$3,614), and Lesotho pledged \$575.

Several of the 'middle income' developing countries agreed to contribute larger amounts. Nigeria, for example, promised

\$250,000, China agreed to contribute \$264,000, and Mexico has indicated that it is likely to make a similar contribution.

Predictably — although with some surprises — the major contributions to the new fund will come from the developed countries. Of the definite commitments made in New York, Italy will contribute over \$9 million in line with its efforts to increase substantially its aid to developing countries, the Netherlands and Sweden will each contribute \$5 million, and Norway, Switzerland and Austria — the host of last year's conference — \$2 million apiece.

Delegates to the pledging conference from both France and Germany indicated that, although administrative reasons had made it impossible so far to earmark specific sums for the new fund, both intended to make contributions of about \$5 million.

Of the other major industrial countries, Britain had made it clear at Vienna that cutbacks in foreign aid made it impossible to commit any new money to development projects (and indeed no British delegation turned up for the pledging conference). Japan said it is still thinking about whether to contribute; and Canada stated that its government had decided to channel all new aid for science and technology in developing countries through the International Development Research Center (IDRC).

The US contribution, initially expected to be considerable, has fallen victim both to general cutbacks in federal expenditure, and to political conflicts that have surrounded Congress's successful torpedoing of the administration-backed Institute for Scientific and Technological Cooperation (ISTC).

In Vienna, US delegates had talked informally of a contribution in the region

of \$50 million. By the Autumn, this had dropped to \$25 million, and when President Carter's budget request to Congress was delivered in January, it had fallen to \$15 million.

Now it looks as if the contribution will be even lower. The US delegation to last week's meeting stated that its contribution would be between \$10 million and \$15 million — and it is thought that the lower of the two is the more likely. In addition, the US attached two conditions to its contribution. Firstly, that it should not be more than 20% of the total fund; and secondly that "significant contributions are made by countries receiving large incomes from oil exports".

Such contributions are expected, but have not yet materialised. Saudi Arabia, announced that it would indeed provide support, but that the precise amount was yet to be decided by its government. And Venezuela, one of the Latin American countries which had been arguing most strongly for new financial mechanisms, surprised many of those present by stating that it, too, while prepared to support the fund, had not yet decided on the extent of its support.

Despite some disappointment that the initial total had not been higher, UNDP said that they were well satisfied with the results of the pledging conference, and are now finalising procedures for deciding how the money should be distributed in a way that supports the final recommendations of the Vienna conference.

In closing the conference, UNDP administrator Bradford Morse said that the contributions received so far meant that the interim fund would be able to operate on 1 May, and several grants are expected to be made soon after that date.

Mr Morse stressed that the contributions promised were only "a first step" towards the target agreed in Vienna, and that a further opportunity for contributions would be provided at a later date.

David Dickson

'Golden Fleece' recipient settles for \$10,000

A MICHIGAN scientist who sued Senator William Proxmire for libel after the senator had awarded a "Golden Fleece" award for the scientist's research on the behaviour of animals under stress has accepted an out-of-court settlement of \$10,000.

The scientist, Dr Ronald Hutchinson, who was previously director of research at the Kalamazoo, Michigan, State Mental Hospital, had received a grant of \$500,000 from the National Aeronautics and Space Administration to support his research. However the grant had been ridiculed by Senator Proxmire as a waste of public funds, stating that the research "should make the taxpayer's as well as (Dr Hutchinson's) monkeys grit their teeth". Last year the Supreme Court ruled that Senator Proxmire could not claim

parliamentary privilege, since the remarks were made not only during congressional debates, but also in press releases and during television appearances.

In making the payment last week, Mr Proxmire issued a statement admitting that some of the statements made about Dr Hutchinson were incorrect. For example, he retracted the claim that Dr Hutchinson's projects were extremely similar and perhaps duplicative, and also that Dr Hutchinson had made a fortune from his research with monkeys. Senator Proxmire and Dr Hutchinson have agreed, following the settlement, that "further litigation is unnecessary". However, an aide to the Senator pointed out that the Senator had not apologised for making the award — and intended to make further "Golden Fleece" awards in future to those whose research projects sounded particularly pointless. □

Sweden

Limited nuclear programme favoured

IN the referendum on nuclear power held on 23 March, 58% of Swedes voted for the completion of the present 12 reactor programme, but only 18.7% gave their approval to anything beyond that.

The referendum was a political exercise from start to finish (see *Nature* 13 March 1980, pages 117-8), and it came as no surprise that the combined forces of the two pro-nuclear options outweighed the support mustered by the single anti-nuclear one. Nevertheless, the success of the anti-nuclear movement should not be underestimated. Against the opposition of three of the four major parties, the trades union movement and industry, it managed — with far less money but far more personal engagement than its rivals — to gather nearly 40% of the voters to its side. The members of the anti-nuclear campaign, led by Lennart Daléus, are unanimous that their fight will go on, although the form of their future organization has yet to be decided.

On the pro-nuclear side, the softer option which recommended carrying out the planned 12-reactor programme but not building any more turned out to be marginally the most popular option of all, polling 39.3%. The more enthusiastically pro-nuclear option polled 18.7%. The fact that nearly 78% of the voters supported options which, with some credibility, demanded phasing out nuclear power, suggests that the population at large accepts nuclear power for a limited period while renewable energy sources are further developed, but by no means wholeheartedly embraces it as the energy system of the future.

Even if this is a reasonable interpretation of the attitude of most people, it remains to be seen whether the politicians will act on it. While they will be under intense pressure to increase funding for renewable energy sources, their immediate tasks will be to bring more reactors on-line. It seems certain that the Ringhals-3 and Forsmark-1 reactors will be quickly loaded: permission to load them has already been given but the actual loading was held up pending the referendum result. The State Power Board has also sought permission to load the Forsmark-2 and Ringhals-4 reactors, but as the reprocessing contract presently in force between the Swedish nuclear fuel supply company (SKBF) and the French firm Cogema does not provide for sufficient capacity to reprocess all the spent fuel from these two reactors, permission to run them will initially be limited to that time which will not produce more waste than the present contract could handle.

If, as the board hopes, Forsmark-2 were loaded in the spring of 1981, and Ringhals-4 in the spring of 1982, the present agreement would take care of

wastes produced from them both until the end of 1985. SKBF is currently trying to persuade Cogema to reprocess an extra 50 tons of spent fuel, which would cover the reactors until the end of 1986. Construction work will also go ahead on the depot for intermediary storage of spent fuel near the Oskarshamn reactors. According to a spokesman at the State Power Board, it seems unlikely that new reprocessing agreements will be concluded in the 1980s, so this depot will have to take care of spent fuel until new reprocessing agreements could be made in the longer term or until a decision were taken to store the waste directly, without reprocessing it.

Anti-nuclear Centre Party Prime Minister Torbjörn Fälldin has no intention of resigning, in spite of the fact that he had

staked all his prestige and moral strength behind the anti-nuclear campaign. His reaction to the result was to say that the people's desire for a maximum of 12 reactors must be respected. This has been seized on by his opponents as an opening shot in a campaign to do away with the twelfth reactor, Oskarshamn-3. The present government is a coalition of the Conservative, Liberal and Centre parties, and such a campaign would be carried on for purely political motives. It would be supported by some sections of the Liberal and Social Democratic parties, but staunchly opposed by the Conservatives' leader, Goesta Bohman, who backed the referendum's most pro-nuclear option. The coalition is undoubtedly in for some tough in-fighting.

Wendy Barnaby



Aldabra has the largest breeding colony of frigate birds (Fregata) in the western Indian Ocean.

Environment

Aldabra faces problems

ALDABRA, the atoll in the Indian Ocean that narrowly escaped becoming an air staging post 13 years ago, once more faces an uncertain future. On 31 March wardenship of the atoll and its ecological treasures passed from the Royal Society, which has maintained a programme of research and conservation since the first alarm, to the Seychelles Islands Foundation. This new public trust has members representing the Seychelles government, the Royal Society, the Smithsonian Institution and international organizations well attuned to the scientific value of an island virtually unscathed by human interference, but its task will not be easy. The foundation will need \$120,000 a year to maintain its research station and scientific programme.

The inhospitability of Aldabra has preserved it from most of the exploitation

suffered by other oceanic islands. The goats, cats, rats and mice introduced after 1880, when a small settlement began on the west island, have not wrought ecological havoc on the other three islands of the atoll. And so when the UK government announced in 1966 its intention to set up a military airfield on the atoll, ecologists realized that they were about to lose a unique ecosystem, where biogeographical and evolutionary theories could be tested.

The Royal Society became the focus for international concern, and mounted an expedition in 1967 to make an inventory of the terrestrial and marine features of the atoll before development began. But in the meantime the plans for the airfield were abandoned, and the society established a more permanent presence. A small research station was built, and in 1971 the society acquired the lease of the atoll, making possible a more comprehensive programme of research, which continued when Aldabra passed into the care of the Republic of Seychelles in 1976.

Since then almost 100 visitors have studied the geology, flora and fauna of Aldabra, making it one of the best known of all oceanic islands and coral atolls. Among the most spectacular of the fauna is the unique colony of about 150,000 giant tortoises, *Aldabrachelys gigantea* (a subspecies of *Geochelone*).

The future of this work is uncertain because the Seychelles Islands Foundation must rely on its own financial resources. In November 1979 it launched an appeal for at least a million dollars to provide an endowment, the income from which could be used to conserve Aldabra in perpetuity. So far more than \$300,000 has been raised, but that will not support the scientific presence much beyond the end of 1980.

Mary Lindley

The World Wildlife Fund, 29 Greville Street, London EC1 8AX, is a focus for the appeal in the UK.

PLANNED changes in Bulgaria's higher education system are designed to provide an annual crop of graduates whose training has been specially tailored to that year's economic needs. Final details of the plan have yet to be worked out, but it appears that at least some of those involved in the new scheme view it with misgivings.

According to Professor Angel Pisarev, Deputy Chairman of the State Committee for Higher Education, the need for the reform has its roots in the pre-war structure of Bulgarian education. At that time the country had no higher technical education whatsoever, and budding engineers had to go to Germany, France or Hungary for their training. Immediately after the war, it was decided to build up a network of polytechnics, giving a degree-equivalent training in such subjects as machine construction, electrotechnics, chemical engineering (including food technology), building and architecture. In all, Bulgaria now has 10 such polytechnics, five of them in Sofia.

Meanwhile, the pure sciences remained the province of the universities, (although a major change in 1972 "integrated" the University of Sofia and the Bulgarian Academy of Sciences, in a scheme by which the Academy took over part of the training of doctoral students, both bodies remaining autonomous).

Measures for implementing the new scheme were approved last year, and an

Eastern Europe

Higher education in Bulgaria

interim system was introduced this academic year to alter the content of lectures and "give more knowledge in the fundamental subjects and a greater practical knowledge at the end", plus an extra 4-5 months of practical education after the academic course but before graduation. The new scheme, to be introduced in 1981, is more radical.

First, the university or polytechnic course, only recently extended from four to five (officially 4½) years, in line with Soviet practice, will be extended to 5½ years. In the first two years, students will receive "fundamental preparation" in fairly generalized courses (physico-mathematical, chemical-biological, electrotechnical, etc.). At the end of that time, they will split up into "general specializations", on the basis of a document drawn up in consultation with factories, agroindustrial complexes and the like. The ministries concerned, having once ordered a certain number of students, will have to find them a job at the end of their

course. There will be no detailed assignment at this stage, since 15-20% of students fail to graduate.

Job assignment will come at the end of the fourth year. Prospective jobs will be posted on notice boards and research posts will be advertised at the same time. During the final year and a half, the student will receive his education partly at the university and partly at his future place of employment. (In the case of a future science teacher, he will do his pedagogics course at this stage.) Finally, after 5½ years the student will graduate and proceed to his appointed job.

According to advocates of the scheme, not only will the new method relieve the student of worries about future employment during their final years; it will also allow the unification of higher education throughout the country. In some years, Pisarev admitted, certain specialisations may be completely closed if no further specialists in those fields are deemed necessary.

Industry, said Pisarev, neither wants to nor can come to terms with the new system — a reaction which may not be unconnected with the fact that industry will have to bear some of the cost of laboratory work in universities and technical training. Bulgaria already has a contract research system involving some students. Now such research will be an obligatory part of the 'basic educational process'. **Vera Rich**

Interferon

Weizmann's interferon entries

THE latest entries for the great interferon race come from an international bevy of owners, headed by the Weizmann Institute in Israel. Not wishing to go nap on a single entry, the Weizmann has simultaneously backed three separate candidates in the hope that at least one will give them a share of the pay-off. Of the three, two involve cell culture techniques and the other embraces genetic engineering.

The genetic engineering project is under the scientific aegis of Professor Michael Revel of the Weizmann Institute and Professor Pierre Tiollais of the Pasteur Institute whose laboratories have considerable experience in interferon and recombinant DNA, respectively. Already their collaboration has resulted in *Escherichia coli* which are producing trace amounts of interferon, an achievement similar to that of molecular biologists in Zurich backed by Biogen (see *Nature*, 24 January 1980, p319) and, before them, Japanese scientists led by Professor Taniguchi at The Yeda Research and Development Company, which handles industrial processes arising out of the Weizmann's research, together with the Pasteur filed an Israeli patent application for the cloning of the human

interferon gene in November 1979.

Yeda is now collaborating with the Cetus Corporation in Berkeley, California in an attempt to improve the bacterial yields of interferon and to scale up the process.

Yeda is also involved in separate attempts to produce fibroblast and lymphoblastoid interferon by cell culture techniques. Professor Revel's team can already do that on a laboratory scale. To scale up production of lymphoblastoid interferon, an arrangement has been made to use the facilities of the Merieux Foundation in Lyons, France. For fibroblast interferon, Yeda and the Swiss Ares Company are building a plant in Israel.

For those who wish to wager a bet, it seems that the Weizmann's recombinant DNA entry is up with the leaders whereas the cell culture candidates are some way behind competitors such as Searle and Wellcome Research Laboratories. There are, however, many dark horses, and undeclared entries to contend with. □

United Kingdom

Science budget steady

A FOUR-year period of relative stability for overall science funding is promised by the

UK Government in its White Paper on Expenditure Plans until 1983-84. But in the division of the 1980-81 science budget the Science Research Council have, on paper at least, received only 52.5% compared with 55% a year ago.

The money available in the 1980-81 science budget will be £383m. It is planned that the budget will be the same in real terms next year and 1% higher in 1982-3 and 1983-4. In announcing this, the White Paper says "The Government attach importance to the support of basic science, in which the United Kingdom excels, as an investment in the country's industrial and intellectual future." Whether or not that sentiment tallies with a four-year static science budget has to be judged in the context of a 9% reduction in expenditure on education planned over the same time.

Of the £383m science budget for 1980-81, the Agricultural Research Council will get £35m, the Medical Research Council £71.8, the Natural Environment Research Council £45m, and the Science Research Council £201.1m with the rest being divided among the Social Science Research Council, the British Museum, Natural History and the Royal Society. Compared to its share of the total a year ago, the Science Research Council appears to have lost out to the Medical Research Council (+1.5%) and the Natural Environment Research Council (+0.7%).

Peter Newmark

NEWS IN BRIEF

No change for US nuclear export licensing

UNDER heavy pressure from the US Congress, President Carter has backed off proposals to remove responsibility for issuing licenses for the export of nuclear fuels and technology from the Nuclear Regulatory Commission (*Nature*, March 20, page 201).

Several members of the administration, including Mr Gerard Smith, Assistant Secretary of State for non-proliferation matters, had lobbied strongly for the change, arguing that the NRC should not be given foreign policy responsibilities. The move was supported by the nuclear industry, which has recently suffered from a poor export performance. However it was firmly resisted by Congress, which argues that in allocating such responsibilities to the NRC under the Nuclear Non-proliferation Act of 1978, it had sought specifically to remove evaluations of the adequacy of safeguards from direct policy considerations. Following President Carter's rejection of the proposed change, an administration official was quoted as saying that the President did not consider it to be "the right time to raise the issue before Congress".

Photocopying company stops multiple copying

THE Gnomon Corporation, a photocopying corporation with branches in eight eastern university towns, including Cambridge (Massachusetts) and New Haven, signed a legal undertaking last week that it would not make multiple copies of portions of any printed material, even if the material does not carry a copyright notice. The company agreed to make the declaration following charges that had been brought against it by eight leading textbook publishers, and backed up by the Association of American Publishers, that material from their publications was being copied without permission, in violation of the revised copyright law of 1978.

The publishers presented evidence against the Gnomon Corporation which they claimed was typical of a huge volume of illegal copying. Each piece of evidence involved the photocopying of at least an entire chapter or complete journal, and in many cases a third to half of a book.

According to the vice-president of the publishers' association, most of the photocopying was of 20-30 copies for university lecturers. The eight publishers filing the suit included Prentice-Hall, John Wiley and Sons, and Princeton University Press.

Gnomon has also accepted responsibility for photocopying done on coin-operated self-service equipment in its stores. The

president of the Corporation, Mr Adam Carley, has said that parts of the agreement are highly objectionable. He considers that his company's copying practices were legal "but we couldn't afford the legal fees to fight the suit".

New committee on recombinant DNA research

AN interdepartmental committee has been established in the US to study the possible health implications of the industrial applications of recombinant DNA research, prompted by the concern of Dr Eula Bingham, Assistant Secretary of Labour responsible for occupational safety and health.

The committee is a subcommittee of the interagency committee on recombinant DNA research, and will be chaired by Dr Gilbert Omenn, associate director of the Office of Science and Technology Policy. The executive secretary will be Dr Bernard Talbot of the National Institutes of Health.

The new committee, which is holding its first meeting next week to decide on a plan of work, has been set three tasks by the interagency committee. First to review the industrial applications of recombinant DNA research. Secondly to assess the need for providing educational information to workers about the techniques they will be coming in contact with. And thirdly to determine criteria for examining the question of potential hazards.

Five-year anti-nuclear campaign launched in UK

FRIENDS of the Earth has launched a £1 million, five-year campaign against the Conservative Government's plan to introduce pressurized water reactors into the UK at the rate of one each year starting in 1982. The campaign aims to turn nuclear power into a major issue of the 1984 general election. It plans to do this through a five-year strategy consisting of increasing public awareness through demonstrations, local elections, and summer schools on nuclear power in the first year; continuing education and initial fund-raising in the second and third years and community organizing and mobilization in the fourth year. The election campaign in the fifth year will support candidates who declare themselves to be against nuclear power.

According to Czech Conroy, FOE campaign director, the programme will emphasize three policy issues — safety, the record of the nuclear industry, and the cost of nuclear power — and three political issues — governmental secrecy on nuclear power issues, the use of taxpayers' funds for pro-nuclear campaigns and the morality of risking nuclear power development without adequate safety and waste disposal facilities.

Research reactor for Bangladesh

THE Bangladesh Atomic Energy Commission recently signed a contract with the US company General Atomic for the supply of a 3-MW TRIGA Mark-II pulsed research reactor. The TRIGA (Training Research and Isotope Production Reactor by General Atomic) is a water cooled, graphite reflected reactor suitable for research training and isotope production. It has a steady state power level of 3 MW and a peak power output of 1,200 MW. The reactor will be installed at the Bangladesh Atomic Energy Commission's research establishment at Savar, about 50 kilometres north of Dacca.

M. Kabir

Givaudan negotiates out-of-court over Séveso

THE Givaudan Company, a subsidiary of Hoffman-La Roche, is negotiating with the Italian government and regional authorities for payment for damages caused by the uncontrolled release of dioxin from its ICMESA chemical plant in Séveso on 10 July 1976. If the Commission of Parliament for Lombardy approves the settlement, Givaudan will pay £24.3 million to the Italian state and the Lombardy regional authority for their expenses incurred in public health, social assistance, damage to the environment, and loss of local industry and trade. Givaudan will also complete at its own expense the decontamination work currently underway.

AUT fights London layoffs

In a letter to Lord Annan, the Vice-Chancellor of the University of London, the Association of University Teachers criticizes the Flowers report on the reorganization of medical education in London (*Nature*, 6 March, page 5) for its potential to make teaching and research staff "the victims of proposals to cure the alleged defects of a system for whose construction they bear no responsibility".

According to John Akker of the AUT, the Flowers report does not say how its proposal to save £3 million from "rationalization" of staff (out of a university budget of £200 million) should be put into effect. The AUT also criticizes the report for not first estimating whether or not savings could be made from sales of buildings and saved maintenance costs and also for not including the cost of redundancy payments and rehousing of staff in expanded facilities at non-threatened institutions.

East-West fencing at Geneva

The outcome of the recent review of biological weapons control does not bode well for other arms control negotiations, writes **Julian Perry Robinson**

THE 1972 Biological and Toxin Weapons Convention (BWC) represents the only real disarmament measure agreed since the World War II peace treaties because it is the only one that has required the destruction of existing stocks of weapons. But, as a model for further agreements, it is weakened by flimsy arrangements for verification. These were the subject of fierce controversy at a conference to review the convention, held in Geneva at the end of last month.

At the one extreme was the Swedish delegation seeking amendment of the treaty to provide for an expert consultative committee under the UN Secretary-General, empowered to investigate complaints of violation. At the other extreme was the Soviet delegation, arguing that since no one had yet raised a complaint under the procedures which the treaty already provided, there was no need to elaborate additional procedures. Between the two was the UK.

Two thirds of the way through the conference, however, there was a turn of events which paradoxically strengthened the position of the USSR. The US State Department suddenly used one of its daily press briefings in Washington to declare itself suspicious of Soviet compliance with the BWC, citing an unexplained outbreak of infectious disease in Sverdlovsk eleven months previously. In various different forms since October, this outbreak has been reported in the British and West German press, with speculation that an accident had occurred in a clandestine biological weapons facility (*Nature*, 27 March, page 294).

The abruptness of the US announcement, and its failure to display any very solid substantiation, strongly suggested that the Americans had launched a wrecking operation, driven by growing anti-arms control sentiments in Washington, and perhaps by the pro-chemical weapons lobby. The drafting party which was then putting together the bones of the final declaration of the conference felt compelled to avoid language which suggested that the BWC had been fully observed, but the US delegation eventually let it be known that it would not raise the matter at the conference until the final plenary session. Since the text of the final declaration should by then have been adopted, this implied that the US delegation was, after all, going to maintain its initial stance of preserving the BWC against damaging aspersion.

The Sverdlovsk allegation very much affected the content of the final declaration

on the thorny issue of the consultative committee. On the one hand it illustrated most graphically the need for some form of international verification procedure. On the other, it suggested that the USSR would be the subject of the first complaint to be brought before the committee, and few states were happy to contemplate the political furor that would ensue, and the attendant threat to the BWC's continuation. The outcome was ambiguous talk about the possibility of a "consultative meeting . . . at expert level" being convened under the verification article of the treaty, without details about who would convene it or what it was supposed to do. Its only merit is that it leaves the question open for further consideration "at an appropriate time". The Soviet delegation had argued that a review conference was not a proper place for amending a treaty — a principle which many delegations subscribed to, including those worried about verification (presumably because of fear of setting a precedent for the 1968 Non-Proliferation Treaty review in August this year).

Some delegations appeared to have taken note of a submission made by the Pugwash movement, which in one of its East-West conferences of senior scientists back in 1959 had set the ball rolling towards international agreement on the BWC. Part of its submission was that highly toxic substances of military potential might well become accessible via certain of the new biotechnologies that are beginning to attract heavy investment, and that if this were to happen it would not be clear whether the substances would be treated as toxins within the meaning of the BWC.

The three co-depositaries of the convention — the US, the USSR and the UK — were reluctant to concede that there might ever be any problems over the scope of the treaty, for this might undermine confidence in it; and they produced an anodyne joint paper on scientific developments since 1972, including DNA hybridization techniques, which concluded that nothing had happened which necessitated any clarification of scope. The conference did not take serious issue with this conclusion. But it decided, largely on the insistence of Sweden, to hold a further review conference sometime between 1985 and 1990.

There are certainly several loose ends hanging out of the BWC which might cause problems as long as there is no analogous convention on chemical weapons to tie them up. This is recognised in Article IX of the BWC, which requires party states to

"continue negotiations in good faith" on chemical weapons. When the operation of this article came under review, many of the delegations strongly criticised the two superpowers for the apparent absence of major progress in their secret bilateral negotiations on the subject (the eleventh round of which was then in session down the street), and for their opposition to the opening of complementary negotiations within the multilateral UN Committee on Disarmament (the CD). Chemical weapons reached the CD agenda soon after the BWC review conference had begun, which seems to have hastened a compromise on the complementary negotiations issue. But apart from the Americans, no one seems at all happy with the compromise, which calls for the convening of an *ad hoc* working group of the CD to "define, through substantive examination, issues to be dealt with in the negotiation on a chemical weapons convention". The primacy of the bilateral negotiations is thus preserved for the foreseeable future, so that the prospects for worldwide prohibition of chemical weapons will remain dependent on the caprices of the superpowers.

The BWC review showed very clearly that the superpowers are in no mood to accommodate one another on chemical or any other arms-control matter, despite growing international tension. The Americans seem set on a policy of brinkmanship threatening (as with the current calls from Washington for an expansion of NATO chemical-warfare capabilities (see page 286)), to destroy the basis of negotiation. Soviet reactions (like their refusal to accept the modest degree of international verification inherent in the BWC consultative committee proposals) are commonly seen in the West as intransigence on basic issues. Neither has the USSR displayed any understanding of the roots of western mistrust. It did not respond to the suggestion that states should voluntarily declare whether or not they had formerly possessed biological weapons.

Many of the participants to the conference regarded the affair as a dry run for the second NPT review conference in August. This attitude no doubt stemmed from the fact that biological weapons make little sense militarily and have never been developed to their full mass-destructive potential. But the chief significance of the meeting lay rather in the influence it might have on other arms control negotiations.

In private conversation about the Sverdlovsk allegation, a Soviet representative doubted whether there could now be any progress in the CD on chemical weapons or anything else at all important this year. Perhaps he was speaking in the heat of the moment — but the present climate does not favour optimism. □

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CORRESPONDENCE

Chinese Scientific American

SIR, — In the second of his three recent articles on the development of science and technology in China (*Nature* 7 February, page 516) Tong B. Tang makes the statement, based on his visit to China at the end of last year, that "There are no cover-to-cover translations into Chinese" of foreign scientific journals. It may interest your readers to know that this statement was not true then and is not now. *Ke Xue*, the Chinese-language edition of *Scientific American*, began regular publication in China with its January 1979 issue, lagging the parent English-language edition by 14 months. Beginning with the January 1980 issue *Ke Xue* will lag the parent edition by only four months. The paid monthly circulation of this "cover-to-cover translation into Chinese" of a foreign scientific journal is currently 25,000.

GERARD PIEL

Scientific American, New York, US

How safe are safety cabinets?

SIR, — There has been much concern about the performance and testing of microbiological safety cabinets built before the publication of the new British Standard (BS 5726) (*Nature*, 278, 29 March, 1979, p.384). This concern is now being extended to cabinets built after the introduction of the standard and claiming conformity with it. Many of the problems are centred on the Class II Safety Cabinet which is designed to provide protection both to the work and the worker. In terms of safety, the two most important features of such cabinets are:

- that pathogenic aerosols are adequately contained by the inflowing air-streams; and
- the reliability of the filtration system is assured.

The BS allows that once the performance of a prototype cabinet has been validated, the subsequent production versions may be assumed to have satisfactory performance, provided that they have the same physical dimensions and that the air-flows do not differ by more than $\pm 10\%$ from those of the prototype. We do not believe that this principle is in practice adequate to provide safe working conditions, particularly in open-fronted Class II cabinets.

We have undertaken trials to evaluate production versions of a number of these cabinets and to correlate different methods of determining operator protection factors. Early in these trials the disturbing fact emerged that several cabinets reputedly identical in design to prototype models which had performed satisfactorily, failed to provide the required operator protection. The monitoring instruments of the cabinets indicated safe working conditions, and it was only from the results of aerosol containment tests that faults were revealed.

Most of the defects like faults in fan motors and their controls, or defective filter seals could have been rectified on site. But, more important, we have found that relatively low air movements in laboratories can severely affect containment; the approved design may not be adequate in such circumstances.

We urge most strongly that commissioning of new cabinets should include tests for containment, filter integrity and the quality of their seals; without these on-site tests there can be no guarantee that the cabinet will perform within specification.

Section six of the Health and Safety at

Work Act places an onus on suppliers, manufacturers and installers to provide cabinets suitable for the use for which they were designed and complying with the requirements of BS 5726. The customer should satisfy himself that these obligations have been met. Such tests must be included in the purchase price and not left as options for intending purchasers.

The DHHS operates an effective evaluation scheme for Class I cabinets, and there is clearly a need for a similar service for Class II types. This should cover all the requirements of the British Standard, but should in our view be supplemented by a test following installation on site. Agencies operating a rigorous approval scheme should be accredited by the British Standards Institution.

The results of the recent trials will be reported in detail in due course. One finding can be mentioned now: namely, that when tests using micro-organisms are unacceptable an alternative method making use of an aerosol of potassium iodide may be a suitable substitute.

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Synergism v. cost benefit

SIR, — In a disturbingly high proportion of experiments on mixtures for pest and disease control, decisions are based on questionable criteria. Many biologists appear to have become convinced that the ultimate goal is synergism. In screening candidate mixtures for further study a mixture that is not considered synergistic is sometimes abandoned.

Certainly, it is desirable to gain understanding of the mechanism of joint action, but often the need is only to assess the net observable effect of applying one mixture or another. The purpose is usually to minimize an overall cost, where the cost or loss function may include not only the cost of materials, equipment and labour, but also the losses in terms of environmental and health hazards, and damage to non-target organisms. On this basis, even mixtures with some degree of antagonism may sometimes be worth considering.

The point at issue is illustrated in Fig. 1, which shows contours of equal effect (isoboles) and of constant cost, plotted against rates of application of the components of a two-component mixture, assuming each component to be individually active. If the cost is linearly related to the amount of each component (Fig. 1a), a mixture can have no advantage over a single material unless the isobole at the required level of response is

sufficiently concave upward. The requirements for upward concavity are more severe if the cost curve is also concave upward (Fig. 1b), but if the cost curve is concave downward (Fig. 1c) even a mixture with a concave-downward isobole might be of practical value. Cost curves such as these can arise, for example, if either component has adverse effects on non-target organisms or on the environment, often the very reason for investigating a mixture.

All this may seem to be so obvious as to need no stating, but mathematically-inclined scientists might be surprised to discover the extent of the primary emphasis on synergism, and the relatively slight or belated consideration of cost-benefits.

Even if demonstration of synergism is considered relevant, there are varied concepts of what constitutes synergism, except in the case where only one component of a mixture is active on its own. I have elsewhere reviewed some of the confusion that exists in one field of application, a confusion that has its roots in failure to realise the importance of defining an appropriate reference model to represent absence of synergism (and antagonism). For example, many weed scientists, using a method due to Colby, consider that any mixture is synergistic if the proportion surviving is less than the product of the proportions that survive the separate components. On this basis, a "mixture" of two aliquots of the same material would be judged synergistic.

Other biologists consider a mixture to be synergistic only if its isobole is concave-up. The models corresponding to absence of synergism by these two definitions are, in general, quite different. Either of them, or neither, may be biologically relevant. Occasionally both definitions are even treated as if synonymous. Another common misconception is exemplified by the "definition" in the *Herbicide Handbook*. "Complementary action of different chemicals such that the total effect is greater than the sum of the independent effects". On this basis, a mixture for which the percentage responses to the separate components sum to more than 100 would always be classified as antagonistic.

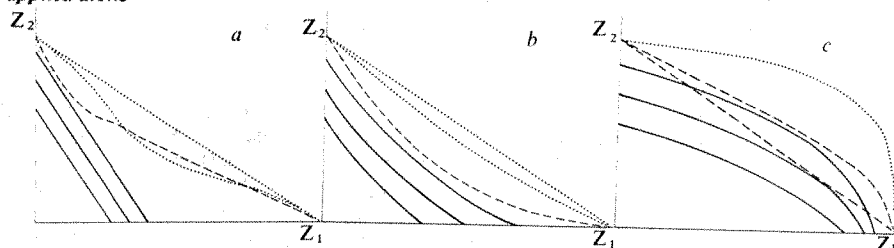
The problems of definition are neither easy to resolve nor to explain and demonstration of synergism in a fundamental sense may require specialized biochemical experimentation.

The aim of this communication is to plead for recognition of the real purpose of any study of joint action, and for appropriate assessment of the results. Cost curves may be difficult to establish in absolute terms, but even a rough idea of their forms could usefully influence the focus of screening and field trials of mixtures.

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Fig. 1 Curves of constant response (broken lines) and curves of constant cost (solid lines) for mixtures of two materials S_1 and S_2 at doses Z_1 and Z_2 respectively. The cost attributable to S_2 has been assumed to be proportional to Z_2 , so that in each diagram any two cost curves are at a constant vertical distance apart. Examples in which a mixture would be economically advantageous have isoboles shown by dashes (---); mixtures with dotted isoboles (....) could not cost less than S_2 applied alone.



NEWS AND VIEWS

Archaean stromatolites and the search for the earliest life

from Euan G. Nisbet

SEARCHING for the oldest traces of life on Earth is a difficult business — if the evidence for the existence of life is good, the rocks are usually not very old; if the rocks are very old the evidence for life is usually not convincing. In this issue of *Nature* Lowe (page 441) and Walter *et al.* (page 443) separately report two probable examples from the Pilbara, Western Australia, of $3.4\text{--}3.5 \times 10^9$ yr old stromatolites (sedimentary structures formed by algae or possibly other organisms). These may be the oldest macro-‘fossils’ yet found. These two reports and other recent work suggest that life began very early indeed in the Earth’s history.

To be convincing, a report of evidence for life in the Archaean (that period before 2.5×10^9 yr ago) must demonstrate both that the structures described are unambiguously biogenic and that the geochronological control is accurate. Cloud and Morrison (*Precamb. Res.* 9, 81; 1979) distinguished three categories of evidence, discriminating between ‘permissive’, ‘presumptive’ and ‘compelling’ evidence for life. ‘Possible’, ‘probable’ and ‘definite’ (as far as any geological evidence is definite) would be another way of putting it. Very little of the evidence for life in the Archaean is ‘compelling’, but there is a considerable body of evidence in the ‘presumptive’ class. Careful geochronological work, in particular by O’Nions, Hamilton and coworkers, by Pidgeon and by Moorbath in various very old terrains is promoting the search for better and older evidence.

Palaeontology in the Archaean takes three main forms: the study of possible microfossils; the analysis of rocks for chemical traces of life; and the study of stromatolites. The identification of Archaean microfossils is often not wholly convincing (Muir *Publs. Geol. Dept., Univ. Western Australia*, 2, 11; 1978). Microstructures which could be fossils have been reported from the Barberton area (3.5×10^9 yr) by Knoll and Barghoorn (*Science* 198, 396; 1977) and from the Pilbara by Dunlop *et al.* (*Nature* 274; 676; 1978), but in their review Cloud and Morrison concluded that the oldest structurally preserved microfossils for which there was conclusive evidence were 2.3×10^9 yr old (post-Archaean). The older examples from Barberton and the Pilbara are ‘possibles’, but the evidence is not conclusive. The second line of research, chemical analysis, includes both stable isotope work and the study of organic compounds in kerogen extracted from Archaean sedimentary rocks. Schidlowski *et al.* (*Geochim. cosmochim. Acta* 43; 189; 1979) studied carbon isotopes in 3.7×10^9 yr old Isua metasediments, from West Greenland. Their evidence suggested that the terrestrial carbon cycle was virtually stabilized at least as early as 3.7×10^9 yr ago — the oldest ‘possible’ evidence for life on Earth. Sklarew and Nagy (*Proc. natn. Acad. Sci. U.S.A.* 76; 10; 1979) have isolated possible remnants of biologically made carbohydrates from the Belingwe stromatolites, Zimbabwe Rhodesia (2.7×10^9 yr old). However, post-depositional contamination is a major problem in this research, especially when early Archaean samples are studied.

Archaean stromatolites are on a much larger scale — they can be ‘hit with a

hammer’, but in some cases it is difficult to distinguish between stromatolites and similar but abiogenic sedimentary structures. At best stromatolites offer ‘compelling’ evidence for the existence of life, since analogous modern structures are well-known and have been studied in detail (particularly at Shark Bay, Western Australia). Thus the evidence reported by Lowe and Walter *et al.* is of especial importance. At present the oldest compelling evidence for life on Earth comes from stromatolites about 2.7×10^9 yr old, especially from the Slave Province, Canada (Henderson *Can. J. Earth Sci.* 12, 1619; 1975), from Steep Rock Lake, Canada (Jolliffe *Econ. Geol.* 50, 373; 1955) and from the spectacular outcrops near Belingwe, (Bickle *et al. Earth planet. Sci. Lett.* 27; 155; 1975, Martin *et al. Precamb. Res.* in the press). Slightly older, and not so well exposed, are the probable stromatolites (Mason & von Brunn *Nature*, 266, 47; 1977) of the Pongola Supergroup, S. Africa ($2.9\text{--}3.0 \times 10^9$ yr old). Stromatolite-like structures also occur in the Barberton area, S. Africa, but they may be of inorganic origin. Thus these two new reports of 3.5×10^9 yr old stromatolites add very significantly to the evidence for the existence of life in the early Archaean.

Lowe describes internally laminated conical mounds, interpreted as stromatolites, in recrystallized evaporites which form part of the Warrawoona Group. Criteria for biogenicity cited by Lowe include morphological similarity with younger stromatolites and similarity of sedimentary environment with later examples. Lowe considers that forms similar to *Conophyton* occur in the Pilbara; if so, this very considerably extends the supposed age range of this type

of stromatolite since the only other Archaean example of *Conophyton* is at Belingwe (Martin *et al. Precamb. Res.* in the press). Walter *et al.* report a single nodular structure, also from the Warrawoona Group. They consider that this structure could not have formed abiogenically and thus identify it as a stromatolite.

How will these two reports be regarded by properly sceptical geologists? 'Probable' is the most likely response. The burden of proof is very great in material as old as this, but it is interesting that 'possible' microfossils of the same age have been reported from the same area by Dunlop *et al.* Further fieldwork may produce more definite evidence. Thus the oldest 'possible' evidence for life is that

from Isua (3.7×10^9 yr old); the oldest 'probable' evidence is from the Pilbara stromatolites reported in this issue of *Nature*; and the oldest 'compelling' evidence from the superb late Archaean stromatolites in Canada and near Belingwe. Nevertheless, the 'probable' evidence from the Pilbara, together with the possibility that the Isua rocks do record biological activity, suggest that life on Earth began very early indeed in the geological record. It is difficult to imagine that the surface of the Earth was hospitable to life before, say, $4.2-4.3 \times 10^9$ yr ago. Thus, if life did originate on Earth, the processes leading up to it (*Proverbs* 8.27) must have happened very quickly indeed. A few hundred million years is not long on a geological time scale. □

oncogenes but promoters or other DNA sequences which, by virtue of their integration, induced expression of transforming genes in the recipient NIH 3T3, a cell line and therefore not strictly a normal cell. A more surprising feature of this work is finding that the DNA from all chemically transformed and normal cells tested transforms recipients with equal efficiency, suggesting that the chemically-induced oncogene expression is transmitted poorly to recipients. The author's explanation is that the chemicals may have mutagenically inactivated a *trans*-acting repressor of a transforming gene; the oncogene will therefore never be expressed in a recipient in which this repressor is functional and the transformation is thus phenotypically recessive.

Somewhat different results have been obtained by R.A. Weinberg's group (Shih *et al. Proc. natn. Acad. Sci. U.S.A.* 76, 5714; 1979) who transfected DNA from 15 chemically transformed cells to test the hypothesis that transformation was due to the mutagenic action of the chemicals. Although these workers apparently used high molecular weight DNA, they found that DNA from 5 of the clones transformed with high efficiency, equivalent to that observed by Cooper *et al.* only with DNA from transformed recipient cells (not surprisingly, secondary transfections by Shih *et al.* with DNA from transformed recipients did not further enhance transformation). DNA from the other transformed clones and controls showed no significant transformation, in line with the observations of Cooper's group.

It is difficult to compare the findings of these two groups, given the different premises with which they prefaced their studies and differences in materials and technique. Indeed, only one cell line, the methylcholanthrene-transformed BALB 3T3 mouse cell MC5-5, was common to both studies and this, in Cooper's hands, yielded poorly transforming DNA whereas for Shih *et al.* it was an efficient transformer. However, it does seem likely that Cooper *et al.* were studying cells similar to those which Weinberg's group regarded as irreproducible inducers of transformation (Shih *et al.* apparently never used low molecular weight DNA for transfection). Moreover, Shih *et al.* provide clear evidence for cell transformation resulting from a dominant mutation. Whether these mutations can be in either or both structural or regulatory regions remains to be determined and these findings must be reconciled with the evidence of Cooper *et al.* that genes from normal cells can also induce transformation. As both groups realise, elucidation of these interesting questions requires the application of molecular cloning techniques to provide large quantities of transforming cell DNA and Weinberg *et al.* (12th Miami Winter Symposium, 1980, in the press) have

Fishing for oncogenes

from John Wyke

SOME 10 years ago the existence of particular genes which might be responsible for inducing cell neoplasia was first suggested. This 'oncogene hypothesis' proposed that the RNA tumour viruses owed their ability to transform cells to transforming genes (oncogenes) which they may have picked up at some time, perhaps from the host cell itself. In the past decade a wealth of evidence has partially validated this idea, for we now know that the transforming genes of many retroviruses contain sequences that are homologous to regions in the genome of normal cells, the virus presumably having acquired this genetic material by recombination between its genome and the host cell. These cellular homologues of viral oncogenes may themselves be oncogenic if expressed at inappropriately high levels (as may occur when their expression is controlled by a retroviral promoter) and neoplasia may simply be a matter of gene dosage. On the other hand they may become oncogenes only after mutation by physical or chemical means or during or after recombination with a retrovirus (there may or may not be an additional requirement for inappropriate expression). These putative cellular oncogenes clearly have a potential importance beyond the interests of tumour virology. We would like to know their function, how they are regulated and whether their expression is important in cancer induced by agents other than viruses, and for these reasons they are attracting increasing attention.

Two strategies for identifying and isolating cellular transforming genes have recently been reported. One uses the technique of DNA transfection in which

naked DNA from a donor cell is introduced into a recipient, inducing a phenotypic alteration (in the case of putative oncogenes the phenotype sought is morphological transformation). Two groups, both with experience in transfecting retroviral DNA, have applied donor DNA from normal and chemically transformed cells to recipient untransformed NIH 3T3 mouse cells. G.M. Cooper and colleagues (this issue of *Nature* page 422) reasoned that normal cells might contain unexpressed transforming genes which, if they integrate adjacent to an active promoter in a recipient cell, could be expressed. They found that high molecular weight DNA ($> 20 \times 10^6$) from both normal and chemically transformed avian and murine cells did not increase transformation of recipient cells above background levels but that fragmented DNA from the same donors, sonicated to molecular weights of $0.3-3 \times 10^6$, transformed recipients at a low level (0.003 transformants per μg DNA). They suggested that fragmentation may have separated potential transforming genes from linked cell regulators and these liberated oncogenes, in a few instances, integrate next to an active promoter in the recipient. This interpretation was supported by using DNA from the transformed recipient as a donor in further transfections, when it was found that the high molecular weight DNA was now a very efficient transformer (0.1-1.0 transformants per μg DNA). This indicated to Cooper *et al.* that the oncogene and its newly-acquired active promoter were now transfected in tandem.

These results provide *prima facie* evidence that normal cells contain potential transforming genes, but one slight caveat is the possibility that DNA from the original donors contained not

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already embarked on this project.

The other strategy for picking up oncogenes uses the penchant of retroviruses themselves for acquiring cell sequences. Essentially, the idea is to passage a non-transforming virus through a cell which is transformed, and thus expressing cell oncogenes, reasoning that recombination between virus and oncogene can occur, giving rise to a transforming virus. This ploy should permit not only stable acquisition of the oncogene but also its efficient transmission in an expressed form, using the virus as vector, readily enabling studies on the oncogene product and its phenotypic consequences. Naturally, a number of groups over the past 10 years have tried to

capture oncogenes in this way, but with limited success. However, Rapp and Todaro (*Proc. natn. Acad. Sci. U.S.A.* 77, 624; 1980) now claim to have developed a method for generating *in vitro* new mouse retroviruses capable of inducing leukaemias, sarcomas or carcinomas. Although they have not yet purified and characterised their new isolates, their protocol looks promising.

These two experimental approaches together provide a rational way to identify and amplify cellular transforming genes and initial results are encouraging. Soon they may enable us to learn a great deal about the variety, structure and regulation of oncogenes, and how their products effect the cell phenotype. □

fragments of virus DNA containing the TK gene. At first it was assumed that the required gene would have to be chemically linked to the TK gene but more recently it has emerged that the small number of 'competent' cells that accept the TK gene will at the same time accept other DNA fragments present in the culture medium (Wigler *et al. Cell*, 16, 777; 1979). Correct transcription and processing of rabbit globin genomic DNA linked to a cloned herpes TK gene and introduced into mouse cells, has been shown (Mantei *et al. Nature* 281; 40; 1979).

But there is a prejudice about using viruses, even small cloned fragments such as the herpes TK gene as vectors or as selective markers for introducing genes into cells which are destined to be reintroduced into man.

A different approach has been taken by Cline *et al.* (this issue *op. cit.*) who have transformed normal bone marrow cells with cellular DNA and have chosen a system in which the selection of the transformed cells takes place *in vivo*, after the treated cells have been introduced into a new host. As a marker of transformation they used the dihydrofolate reductase (DHFR) gene, which if present in a large number of copies per cell confers resistance to the anti-cancer drug methotrexate, which acts by inhibiting DHFR. Selection *in vivo* is by subsequent treatment with methotrexate. This selective system allows them to use normal bone marrow cells as the recipients rather than a mutant cell line.

A few years ago R. Schimke and colleagues at the University of California, Los Angeles found that resistance to methotrexate is sometimes a result of an unusual amplification of the genes for DHFR, with consequent overproduction of the enzyme which overcomes the inhibition. As their source of amplified DHFR genes, Cline *et al.* took total DNA from a line of mouse cells in which the DHFR genes had been amplified some 30 times.

They used this to transform normal mouse bone marrow cells by a conventional calcium precipitation technique. The treated cells were injected into mice of a different but compatible strain which had been irradiated to clear the marrow of host bone marrow cells. The treated cells could be distinguished from those of the host by their distinctive chromosome pattern. Along with the treated cells were injected an equal number of 'mock' transformed host-type bone marrow cells treated with DNA from non-resistant cells.

The mice were then treated with methotrexate so that injected cells which had received large numbers of DHFR genes and were able to express them would be selected over normal host or donor cells, and, if stem cells, would multiply preferentially. If there is no selection for the added gene, both types of cell will remain in equal numbers, as the only

Reintroduction of genetically transformed bone marrow cells into mice

from Bob Williamson

MANY human hereditary diseases are caused by the malfunction of a single gene coding for a protein, of which the human haemoglobinopathies are the best characterised. Methods based on recombinant DNA techniques now allow fetuses with these disorders to be identified more reliably and easily. Antenatal diagnosis with the possibility of offering abortion is now a reality for several of the haemoglobinopathies. Another (or complementary) approach to the problem of these genetically simple diseases would be gene replacement, in which a normal copy of the deficient or abnormal gene is introduced into the affected tissue after birth. So far this approach remains firmly in the realms of speculation but recent developments in the introduction of genes into mammalian cells are bringing it nearer. The most recent report from M.J. Cline *et al.* at the University of California, Los Angeles (this issue of *Nature*, page 422) concerns the reconstitution of bone marrow in mice with normal bone marrow cells into which a drug-resistance gene has been introduced *in vitro* and the demonstration that cells which are expressing that gene can be selected by treatment *in vivo*.

The initial problems to be faced in developing methods of gene replacement are first, to introduce the required gene into the appropriate cell *in vitro* and to build into the process some way of easily recognising and selecting transformed cells in which the gene is functional. Second, an

effective population of treated cells has to be reintroduced and maintained.

Attempts to transform mammalian cells with fragments of DNA (transformation here means the introduction of DNA into a cell with a consequent change in its phenotype and does not refer to neoplastic transformation) come up against several difficulties. Mammalian cells will accept 'foreign' DNA although normally at very low frequency and there is some evidence that it may be integrated into their chromosomes. The low frequency of transformation is partly because techniques for introducing DNA into cells in culture are rudimentary, but also because only a small proportion of cells in a population appears 'competent' to accept DNA from solution. It is also difficult to find appropriate mutants of mammalian cells which allow selection for newly-introduced gene function.

One way of introducing DNA into mammalian cells, which provides both a selective marker and a higher rate of introduction, is to use modified versions of certain DNA viruses such as SV40 and polyoma as vectors. If the inserted gene is in the correct orientation and near to a functional viral promoter RNA is made from the inserted gene as well as from the virus and genes inserted into these vectors can be properly transcribed, processed and translated (see for example Mulligan *et al. Nature* 277, 108; 1979; Hamer & Leder *Nature* 281, 35; 1979 using β -globin DNA).

Another approach, taking the herpes virus as the starting material is to use the virus thymidine kinase (TK) gene as the selective marker for transformation of TK-deficient cell lines by genes linked to

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difference in their lineage is the treatment of one with DNA containing large rather than small numbers of DHFR genes.

It turned out that after 30 days, the proportion of marrow cells derived from the DHFR-gene treated marrow culture was greater than 70% in most cases. The proportion continues high as long as methotrexate is administered, but when it is withdrawn the ratio returns to normal. Since the increase was also found in stem cells, this result is puzzling unless one assumes that the DHFR genes are gradually eliminated from the cell population when selection is removed. It is not yet known whether the DHFR genes have become integrated into the cellular DNA; if they are persisting in an episomal state they may be more easily lost. The mice were apparently healthy, as the levels of methotrexate used for selection were not very high. Direct analysis of the marrow cells showed a high level of the enzyme, as would be expected.

One cogent objection that could be raised to this work is that as genes for DHFR do exist in normal marrow cells, it is possible that the resistant cells could have arisen in the treated cells by the amplification of these DHFR genes rather than by the introduction of genes *in vitro*. Indirect supporting evidence that their procedure does lead to transformation and selection of transformed bone marrow cells is provided by other experiments by the same authors (submitted for publication elsewhere) in which cloned herpes TK gene sequences were inserted into mouse marrow cell cultures and these cells were transplanted into irradiated mice of a different chromosome pattern, but otherwise genetically compatible. Unlike the mouse DHFR genes, these herpes TK genes can be unambiguously recognised by their DNA restriction pattern as they are from a human virus and do not normally occur in mouse cell nuclear DNA.

The transplanted marrow did indeed contain herpes TK genes, and these appeared to be integrated in some fashion into the host cell DNA. Moreover, if the mice are treated with methotrexate (which also can select for thymidine kinase by another mechanism) the cells containing the gene are selected for in the marrow.

Bone marrow is at present the only tissue in which one might seriously consider clinical gene replacement in the near future, to correct some of the haemoglobinopathies, for example sickle-cell anaemia. Bone marrow can be relatively easily extracted, manipulated *in vitro* and replaced.

However we do not yet have the answer to the crucial question of whether it will be possible to restore correct control over the introduced genes, for it is absolutely essential that they should be expressed under the same controls as those in normal animals. Expression in inappropriate tissue or abnormal control in the cell in which they are normally expressed could be disastrous. A surplus of one globin chain

would be as harmful physiologically as a deficit, substituting an iatrogenic illness for a genetic one. Before gene therapy becomes a real possibility either ways must be found to insert the required gene into the 'correct' place on the chromosome or other methods

must be found to ensure balanced gene expression.

The final question is, why bother, particularly as antenatal diagnosis is now possible for sickle cell disease and thalassaemia? But there are still many people suffering from these diseases, and there will always be some new ones arising — this in itself justifies the approach. However, the main reason is perhaps that the haemoglobinopathies are quite unusual amongst genetic disease in that carriers can be identified easily. There are many conditions where carriers cannot be detected antenatally, and in this case treatment rather than prevention may be the only feasible strategy. □



100 years ago

The *Photographic News* is responsible for the following:— Everybody knows how jealously the gates of the Royal Observatory are guarded, and what difficulties even scientific men have to gain admission. But Mr. Glaisher, the worthy President of the Photographic Society, and who was until lately Superintendent of the Meteorological Department, tells a story that goes far to prove that nothing is impossible to a resolute man. A vast star shower had been anticipated and its coming heralded in every newspaper. The staff at Greenwich, with the Astronomer-Royal at their head, remained the whole night through making observations and counting the bright meteors as they fell. The weary night passed, and the small hours of the morning came, only to find the jaded observers still pursuing their duty. "That makes 10,704," said our friend Mr. Glaisher. "Beg pardon; how many?" exclaimed a voice behind him. "10,704," repeated the President of the Photographic Society; and then, not recognising the voice, he turned and saw a stranger: "Who are you, and where do you come from?" At first, the only possible conjecture was that the stranger had fallen from the clouds along with the star shower; but it was not so, for, closing a little note-book, he simply replied, "I am the special correspondent of the *New York Herald*. Thank you very much. Good morning." How that special managed to get through the park gates and elude the vigilance of the keepers; how he got inside the walls of the Observatory; how he pressed into the sanctum of the Astronomer-Royal is a mystery to this day; but within a few hours of his interview with Mr. Glaisher the readers of the *New York Herald* printed a correct account of the marvellous star shower, together with many interesting details of the Observatory itself.

The French Exploring Expedition for the Trans-Saharan Railway has left Wargla for the interior of Africa.

From *Nature* 21; April 1, 524; 1880.

Winds in the polar thermosphere

from Henry Rishbeth

THE observations of Smith and Sweeney, reported in this issue of *Nature*, page 437 make a new contribution to knowledge of the polar upper atmosphere — specifically the thermosphere, at heights of 200–300 km. Working in Spitzbergen, during continuous winter darkness, Smith and Sweeney used a Fabry-Perot interferometer to measure doppler shifts of the red 630 nm auroral emission. The instrument points sequentially in different directions and, with a few minutes' integration, typically measures the wind component in the line of sight to within about $\pm 20 \text{ m s}^{-1}$.

The 630 nm ($^3\text{P} - ^1\text{D}$) emission is radiated by the metastable ^1D state of atomic oxygen, which is excited by dissociative recombination of O_2^+ ions or by soft electron precipitation. With a radiative lifetime of 110 s, the ^1D atoms may be expected to take up the neutral air velocity so that the doppler shift of their radiation should correspond to the neutral air wind velocity. The technique is now well-established for measurements of nighttime thermospheric winds at mid-latitudes^{1,2}; more recently the Mawson Institute group in Australia has reported daytime measurements of thermospheric winds with the technique, obtained despite the intense background of scattered solar radiation³.

This optical technique has the drawback that the radiation originates over a range of height, within which the wind velocity may vary considerably. This problem may be real for the thermally-driven winds at lower latitudes; but in the polar thermosphere (as discussed later) the wind is mainly driven by a large-scale electric field which is almost height-independent above 200 km,

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below which there is little 630 nm emission. There might be localized winds in the vicinity of active auroral forms, but most of the Spitzbergen observations were not made in such conditions. The height variation of 630 nm emission can to some extent be studied by using a limb-scanning interferometer carried in a satellite, for example OGO-6 (ref. 4); but only at the expense of horizontal resolution which is several hundred kilometres, at best. The satellite technique does however give the benefit of global coverage, within orbital limitations.

Smith and Sweeney's observations were made in the polar cap, a region usually about 30° in diameter, bounded by the 'auroral oval'⁵ in which auroral phenomena occur. Their results confirm a picture of polar thermospheric winds that has hitherto been based partly on observations by various techniques and partly on theory. Kohl and King⁶ first described the dayside-to-nightside winds across the polar cap that would result purely from heating of the dayside thermosphere by solar extreme ultraviolet (EUV), taking into account the effects of coriolis force and ion-drag (friction between ions and neutral air) on the wind direction. However, intense localized heating in the auroral oval — due partly to particle precipitation but mainly to joule heating by auroral electric currents^{7,8} — must modify the solar-driven wind pattern. Further modification results from electric fields, which at heights above 150 km produce Hall drift of the plasma ('E × B' drift), the horizontal component of this drift being imparted by ion-drag to the neutral air^{9,10}. This electrically-driven wind can be suppressed by 'back pressure' if it creates accumulations or depletions of air anywhere¹¹; however, in the polar cap the wind is nearly divergence-free and little 'back pressure' should occur¹².

The high-latitude electric fields comprise, first, intensive fields of magnetospheric origin, more or less localized in the auroral oval and peaking during substorms, and second, the

extensive dawn-to-dusk field in the polar cap that derives from the streaming of solar wind past the magnetosphere. The latter causes a dayside-to-nightside flow of ionospheric plasma across the polar cap, forming part of a great convective system with return flows at somewhat lower latitudes^{13,14}.

This 'plasma convection' has been studied in some detail by tracking chemical tracers released from rockets^{15,16}, by satellites such as Atmospheric Explorer¹⁷, and by incoherent scatter radars¹⁸; while the associated electric fields has been measured from balloons¹⁹. Smith and Sweeney's data show that the neutral air moves in a similar way to the plasma, at least for the winter conditions they studied. Around midday and midnight, the directions could probably be fitted by the Kohl and King theory⁶ based on solar EUV heating, but the convection theory¹⁵ accounts much better for the wind directions in the morning and evening sectors.

There remain many interesting problems to be resolved, not least the effect of the interplanetary magnetic field configuration on the plasma convection¹⁹. As to forthcoming activities, the previously-mentioned effects of auroral electric fields and joule heating will be intensively studied in the international campaign aimed at quantifying the upper atmosphere's energy budget, to take place in Scandinavia in winter 1980/81. Auroral motions will be increasingly studied with coherent radars such as STARE (the Scandinavian Twin Auroral Experiment). The European Incoherent Scatter (EISCAT) radar will come into operation in Northern Scandinavia and plans exist for upgrading the radar at Millstone Hill (Massachusetts) and moving the Chatanika (Alaska) radar to Greenland. Moreover, the Antarctic thermosphere differs significantly from the Arctic — largely because of the different magnetic field geometry — and future investigations may increasingly be directed southward. □

Response of plants to sulphur dioxide

from J. N. B. Bell

RECENT years have seen a reduction in the levels of SO₂ that are generally believed to inhibit plant growth. Previously it was commonly accepted that reductions in growth only occurred when relatively high concentrations ($\geq 500 \mu\text{g m}^{-3}$) caused visible injury to the foliage. It is now clear that the atmosphere in some sites where the mean SO₂ level is typical of many parts of Britain can substantially reduce the growth of grass species without any obvious symptoms on the leaves (Crittenden & Read *New Phytol.* **83** 645; 1979). Much air pollution research now concentrates on fumigation experiments designed to simulate such low SO₂ concentrations over prolonged periods and is aimed at quantifying the economic impact on vegetation of the 5.8×10^6 tonnes of SO₂ emitted annually in the United Kingdom. In practice it has proved difficult, even for the most intensively studied species, *Lolium perenne* (perennial ryegrass), to establish clear dose-response relationships and furthermore, different groups of workers have produced contradictory results with similar doses of SO₂ (see Bell *et al. New Phytol.* **83**, 627; 1979).

It is becoming increasingly apparent that the results of air pollution experiments are influenced considerably by environmental conditions within the fumigation chambers and there is circumstantial evidence that

slow growth enhances the toxic action of SO₂ (Bell *et al. op cit.*, 1979; Cowling & 'Lockyer *J. exp. Bot.* **108**, 257; 1978). Davies (this issue of Nature, page 483) provides the first unequivocal evidence that environmental conditions causing slow growth can interact with a moderate level of SO₂ to reduce yield.

Phleum pratense (timothy) was fumigated with $343 \mu\text{g m}^{-3}$ SO₂ for 5 weeks under two light regimes, representing summer and winter conditions, respectively. The SO₂ treatment caused a 50% reduction in dry matter compared with clean air controls under the winter conditions, but no effect under the summer light regime. Davies points out that many fumigation experiments are conducted under optimum growth conditions and that extrapolation to the field where there are marked environmental fluctuations, must be treated with caution.

Much of the early work with SO₂ was done in chambers with low rates of air movement, in which relatively high concentrations of SO₂ were reported to be harmless to plants. Ashenden and Mansfield (*J. exp. Bot.* **28**, 729; 1977) questioned the validity of these experiments, by demonstrating the importance of wind-speed in controlling uptake of SO₂ by vegetation. Using wind tunnels, they showed that $315 \mu\text{g m}^{-3}$ SO₂ reduced growth at a wind-speed of 25 m min⁻¹, but not at 10 m min⁻¹. They suggested that the lack of effect at the low wind-speed was caused by a failure to overcome the

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boundary-layer resistance, so reducing the flux of SO_2 to the leaf surface. At the higher wind-speed, which is more representative of field conditions, the boundary-layer resistance was overcome and the stomatal resistance became the main factor controlling SO_2 uptake.

Another problem in fumigation experiments arises from the difficulty in defining and simulating the most important characteristics of ambient air pollution. Most experiments have used a constant concentration of SO_2 , but it is possible that occasional short-term peaks, which occur in the field, may be responsible for yield reductions observed at mean ambient concentrations which are not generally considered toxic. Furthermore, the presence of other pollutants in ambient air may have positive or negative interactions with the effects of SO_2 on plant growth. Thus Brough *et al.* (*Chem. Ind.*, 21 Jan; 1978) reported a 40% reduction in grain dry weight of barley raised in chambers ventilated with ambient air containing a mean concentration of $61 \mu\text{g m}^{-3} \text{SO}_2$, compared with clean air. The proximity of a large brickworks to their experimental site probably resulted in occasional peaks of SO_2 considerably in excess of the mean, as well as the presence of atmospheric fluoride pollution.

Few attempts have been made to relate physiological effects of SO_2 to growth reductions in the field. Recently, Black and Unsworth (*J. exp. Bot.* 114, 81; 1979) have shown that only $50 \mu\text{g m}^{-3} \text{SO}_2$, a level characteristic of many rural areas, can induce reductions in net photosynthesis

and changes in stomatal resistance of *Vicia faba* (broad bean). They have further demonstrated (*J. exp. Bot.* 116, 473; 1979) that the reduction in net photosynthesis at low irradiances was independent of SO_2 concentration. On this basis it was predicted that a fully developed canopy of *V. faba*, in which the lower leaves are shaded, would show a fairly similar reduction in dry matter production, irrespective of SO_2 concentration within the range $100\text{--}500 \mu\text{g m}^{-3}$. This emphasizes the importance of a clear understanding of the influence of SO_2 on whole crop physiology when predicting effects in the field.

Work during the past decade has laid the foundations for the understanding of the long-term effects of low concentrations of SO_2 on the growth of plants and the various factors which may modify these. However, the economic impact of SO_2 injury on plants remains uncertain. There is clear evidence for the stimulation by SO_2 of plant growth on sulphur deficient soils (Cowling & Lockyer, *op. cit.*) and for the evolution of SO_2 tolerance in grass populations in polluted areas (Horsman *et al.*, *J. exp. Bot.* 30, 495; 1979). Research during the next 10 years should be aimed at quantifying the importance of SO_2 or crop growth in areas with different pollution characteristics. Field investigations must be backed up by laboratory fumigations, which should closely simulate the ambient situation with respect to climatic conditions, realistic fluctuating levels of different mixtures of pollutants, and normal practice in crop cultivation. □

reconstructed as an extensive structure like that of bats, involving also the hind limbs and tail, and the weak flight muscles would have been quite inadequate to flap this wing. Physiology and structure combined to suggest a clumsy creature that could only have soared on its great flight membranes. Furthermore, pterosaurs also seemed to have been condemned to a perpetually wandering Flying Dutchman existence, for their weak hind-limbs made it difficult to understand how they could have risen into the air once they had landed.

Research over the past 10 years has overturned all these assumptions and, though many problems remain, pterosaurs now seem rather more comprehensible. Earlier workers had seen hair-like markings on the body, but it took the discovery of a much better-preserved specimen (Sharov *Trudy pal. Inst. Akad. Nauk. SSSR* 130, 104; 1971), together with a much more open-minded approach to the physiology of extinct reptiles, before it became respectable to consider the possibility that pterosaurs were insulated and, perhaps, homiothermal.

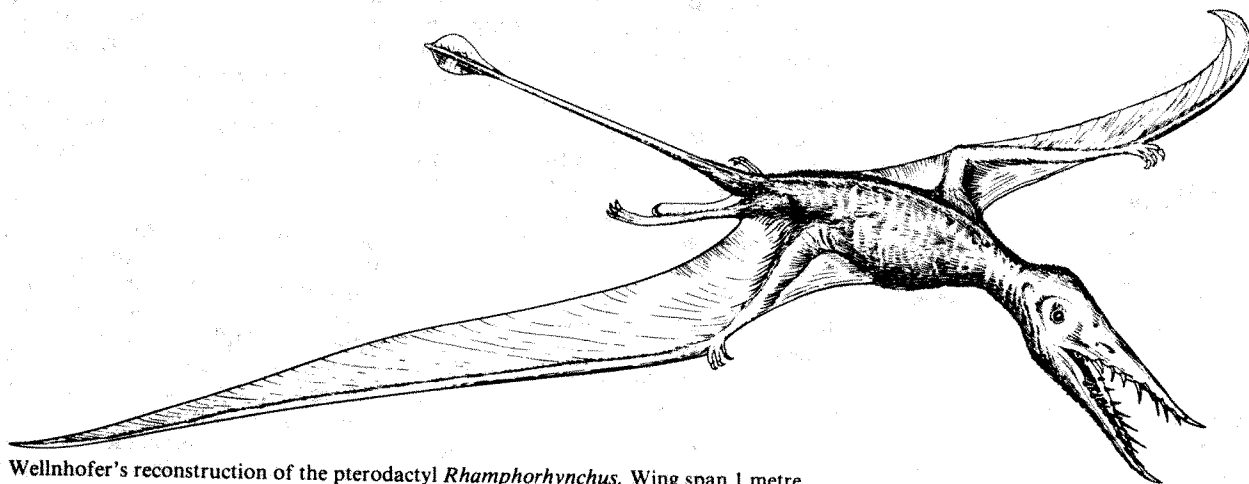
Studying the skeletons and wing-impressions of the smaller pterosaurs, known as pterodactyls, Wellnhofer (*Abh. bay. Akad. Wiss.* 141, 1; 1979) showed that there was no evidence that the wing was attached to the hind foot. He therefore restored the wing with a much narrower, bird-like outline (see illustration), and Padian (*Discovery* 14, 20; 1979) has recently shown that, if the estimated body-weight of a pterosaur is compared with the body-weight : wing area relationships of living animals, the results also suggest a smaller, more slender flight membrane. Such a wing, freed from attachment to the hind limb, is obviously more manoeuvrable in flight and less cumbersome when the wing is folded and the animal is moving on the ground. Wellnhofer also dismisses the belief that there was a flight-membrane between the hind legs and the tail. The resulting slim-line pterosaur will necessitate the reworking of previous calculations on their wing-loading and aerodynamic capabilities (for example Bramwell & Whitfield *Phil.*

Trimming the pterosaur's wings

from Barry Cox

THE old-fashioned view of the extinct flying reptiles known as the pterosaurs seemed reasonably consistent. Being reptiles, they were 'obviously' cold-

blooded. The lack of any high keel running along the sternum suggested weak wing muscles. The flight membrane, borne on the arm and elongated fourth finger, was



Wellnhofer's reconstruction of the pterodactyl *Rhamphorhynchus*. Wing span 1 metre.

THE recent papers by Gleadow (*Nature*, 284, 225; 1980) and McDougall *et al.* (*Nature*, 284, 230; 1980) may have ended a decade of controversy over the age of the KBS tuff in the East Turkana region of Kenya. The KBS tuff is critical to the dating of Early Man and his tools in this important anthropological site. Efforts to date this tuff provide a fascinating and instructive case history of geochronology which illustrates the process of trial and error by which science progresses. Some of the highlights of this case history are sketched below.

The KBS tuff lies near the middle of the Koobi Fora Formation, which yielded its first hominid fossils to Richard Leakey's expedition in 1968. By 1973, 110 hominid fossils had been collected. Primitive artefacts were discovered in 1969, and two occupation sites were excavated in the KBS tuff by Isaac *et al.* (*Science* 173, 1129; 1971). Feldspar from the tuff was dated by Fitch and Miller (*Nature*, 226, 226; 1970) both by the conventional (total degassing) K-Ar method and by the newly developed $^{40}\text{Ar}/^{39}\text{Ar}$ step-heating method. The conventional method produced a wide scatter of ages, clearly in part because of contamination by older feldspar. An age of 2.61 ± 0.26 Myr was assigned to the tuff on the basis of the $^{40}\text{Ar}/^{39}\text{Ar}$ age spectra. Dates younger than 2.6 Myr from the conventional method were believed to reflect argon loss caused by regional hydrothermal alteration.

Doubt about the date of 2.6 Myr was raised by some palaeontologists, who found an age of 2 Myr more in accord

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The KBS tuff controversy may be ended

from R. L. Hay

with dated faunas at Olduvai Gorge and the Shungura Formation in the Omo basin (Cooke & Maglio in *Calibration of Hominoid Evolution* (eds Bishop, W. E. & Miller, J. A.) 303, Scottish Academic Press; 1972). The palaeomagnetic stratigraphy of the Koobi Fora Formation was now studied by Brook and Isaac (*Nature* 234, 344; 1974) with the object of dating the strata by matching their polarity with the newly established polarity time scale. They found two possible fits with the polarity scale, one of which was compatible with the KBS tuff date of 2.6 Myr. Curtis *et al.* (*Nature*, 258, 395; 1975) added to the controversy by reporting K-Ar dates of 1.60 Myr for the KBS tuff at one locality and 1.82 Myr from the tuff at another locality. Their results were particularly significant because dates on feldspar and glass were concordant, thus providing strong evidence against loss of radiogenic argon. If their results were accurate, the KBS tuff must be in fact two tuffs, separated in time by 200,000 years.

Next, zircons from the KBS tuff were dated by the fission-track method. The zircons contained low fission-track densities, but an age of 2.44 ± 0.08 Myr was obtained by Hurford *et al.* (*Nature* 263, 738; 1976). Fitch *et al.* (*Nature* 263, 740; 1976) recalculated their step-heating results and now suggested 2.42 Myr as the best K-Ar age.

In 1977 Harris and White (*Science* 198,

13; 1977) concluded from their studies of suid evolution that deposits below the KBS tuff were equivalent to fossiliferous beds in the Omo basin and Olduvai Gorge that had been dated at about 1.8 Myr. Hillhouse *et al.* (*Nature* 265, 411; 1977), in another palaeomagnetic study of the Koobi Fora Formation, found that although two correlations were possible with the polarity time scale, placing the KBS tuff in the Olduvai normal event (~ 1.7 – 1.85 Myr) created fewer difficulties in the geological history than did an age of 2.4 Myr for the tuff.

Cerling *et al.* (*Nature*, 279, 118; 1979) now correlated the KBS tuff with tuff H₂ (~ 1.8 Myr) of the Shungura Formation on the basis of chemical composition of glass and feldspar. This correlation received strong support from additional dating of the KBS tuff by Drake *et al.* (*Nature* 283, 368; 1980). They gave an age of 1.8 ± 0.1 Myr for the tuff, and explained that the date of 1.60 Myr reported earlier for the tuff at one locality was incorrect because of an error in K analyses.

The latest and perhaps the final statements on the age of the KBS tuff are provided by McDougall *et al.* Their meticulous K-Ar dating of feldspar gave an age of 1.89 ± 0.01 Myr. Fission-track dating by Gleadow yielded an age of 1.87 ± 0.04 Myr, and made a notable contribution to the methodology of fission-track dating of zircons which contain low track densities. In closing, it should be emphasized that the KBS tuff has been a testing ground for various geochronologic methods, both new and old, and the science of geochronology has learned much from the "KBS tuff controversy."

Trans. R. Soc. B 267, 503; 1974). These studies have involved the great albatross-like soaring pteranodontids of the Cretaceous, such as a 7 m wing-span *Pteranodon*, but even this is dwarfed by *Quetzacoatlus* from Texas, the humerus of which is 70% longer than that of *Pteranodon*, and which may have had a wing span of 15 m!

Modern workers seem agreed that the smaller pterosaurs, at least, were actively-flapping fliers, and it now appears that there was, after all, a strong ventral keel projecting forwards from the sternum, which was itself also strongly braced from the scapula by powerful coracoid bones, very much as in birds.

The freeing of the hind-limb of pterosaurs from the flight membrane also helps in the interpretation of their method of launching themselves into the air. Wellnhofer (*Palaeontographica* A149, 1; 1975) believes that the hind-limbs could at least have raised the body into the air far enough that, facing into the wind, the outstretched wings could lift the body from the ground, and that the hind-limb could

also have helped to thrust it skywards.

But further problems arise from the diet of pterosaurs. Both their common presence in marine deposits, and their preserved stomach contents, show that most of them fed on fish. Wild has most recently discussed the problems of the method they used to catch their prey (*Boll. Soc. pal. Italiana* 17, 176; 1978). Though some living birds catch fish from near the water surface by flying close above it, Wild points out that pterosaurs must have been far less competent aerodynamically. This, together with their proportionately much longer wings, would make it inevitable that a wing-tip would sooner or later hit the water, bringing the whole creature down into the sea. Wild also argues that the braking effect of dipping the beak into the water to catch fish would cause such a drop in speed as to bring the pterosaur down. He instead believes that they fished by folding their wings and diving into the water. (This would inevitably have placed considerable strain on their skull and skeleton, and it

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would be instructive to investigate whether they show any convergences with the adaptations of living diving birds such as gannets.)

There is evidence that the hind feet of pterosaurs were webbed and they must have used these for swimming, whether their immersion was voluntary or accidental. Wild thinks it possible that they might have been able to rise from the water surface by spreading their wings, or that they might instead have swum to nearby land so as to rise from a solid surface. (Could it be that the hairy covering had a water-repellent function?)

The pterosaurs which Wild describes come from the Late Triassic and include members of two different families. The origin of the group as a whole must therefore lie in the earlier Triassic at least. Wild points out that the whole organization of the early pterosaurs suggests derivation from a small running and climbing insectivorous reptile. He believes that this strongly suggests that they evolved from the little eosuchians of the Late Permian and Early Triassic, rather

than from the larger terrestrial carnivorous pseudosuchians, most of which were in any case too late in time to be potential ancestors.

Though some problems have then, at

least been tackled, one still cannot help feeling that just as a camel is said to be a horse designed by a committee, the pterosaur is the result of that committee turning its attention to birds. □

Fish vision discussed

from a Correspondent

THE thirty thousand or so living species of fishes inhabit a wider variety of visual environments than any other vertebrate group. Different water bodies vary widely in their colour and turbidity, presenting very different visual tasks to their inhabitants; many species migrate during their life span exposing themselves to these various environments; and there are also often marked seasonal changes in the water's quality. The fishes, therefore, form an ideal group for comparative studies of vision. If we add to this the fact that they are readily available and excellent subjects for physiological experiments, it is not surprising that a great deal of work has been done on various aspects of their vision. Some was reported at a recent meeting of the British Photobiology Society held in London*.

Anatomical papers on the retina (H.-J. Wagner, Universität Ulm) and on cone photopigments (J.N. Lythgoe, University of Bristol) made full use of the comparative approach, and showed that striking differences, clearly associated with the environment, may be found. Explaining their significance is, however, often more difficult. Why, for example, should fish living near the surface be less sensitive to long wavelength light than those that live deeper? They clearly are, though no one knows the reason.

K.H. Ruddock (Imperial College, London) presented results from experiments involving strong bleaching of the retina with a laser, a technique that can reveal receptor interactions that are normally concealed. R. Weiler (Universität München) then described his results on horizontal cells, which posed the unanswered conundrum of how a graded potential can be transmitted without degradation along 600 µm of axon when theoretical considerations suggest 150 µm should be the maximum possible distance. M.B.A. Djamgoz (Imperial College, London) and S.H. Reynolds summarized their work on possible retinal transmitter substances. A wide variety of possible transmitters exist: a list of some 30 candidates was given, of which the most likely ones are GABA and aspartate. Although, however, the evidence is very suggestive, even in these cases it is not conclusive and much work, which should

prove very profitable, clearly remains to be done.

These retinal studies used fish mainly because of their experimental convenience. Reports on the anatomy and physiology of the optic tectum followed in which fish were also used largely for this reason. In the complex analyses that the tectum carries out, however, the problems of the aquatic environment also began to reappear. For example, tectal units responding in a most striking way to very short wavelength light were described for the perch, which is surprising in a fish that does not possess blue cones, has a yellow cornea, and lives in

coloured water in which short wavelength light is almost totally absent. Other units that respond preferentially to repeated stripes were described for this species: could these be related in any way to the fact that the perch itself is striped?

Two papers reported laboratory behavioural studies on spectral sensitivity in fish. W.R.A. Muntz (University of Stirling) reported data that compared vision in an upward direction with vision downward: these showed marked differences in both absolute sensitivity and the form of the spectral sensitivity curve, which may plausibly be related to the difference in the amount of light reaching the fish from the two directions. C. Neumeyer (Institut für Zoologie, Mainz) presented spectral sensitivity data for goldfish. One striking and confusing fact about behavioural studies is that whenever the experimental situation is changed the results also usually change, and these results were no exception to this rule. Presumably different neurones, with different sensory inputs, contribute preferentially to different behaviour patterns. □

Polarity of spindle microtubules

from Jeremy Hyams

UNLIKE microfilaments in which directionality can be visualised directly by specific interaction with heavy meromyosin, the polarity of microtubules has, until now, been approachable only by more devious methods. The most successful has exploited the ability of tubulin subunits with attenuated capacity to self-assemble, to polymerize *in vitro* onto some preformed seed or organizing centre. Various cellular structures will apparently serve although most frequently used are flagellar axonemes from the green alga, *Chlamydomonas*, whose two ends are morphologically distinguishable. When axonemes are incubated with low concentrations of brain tubulin (<2 mg ml⁻¹), microtubules grow by the addition of subunits onto the distal end only. At higher tubulin concentrations, growth is onto both ends of the axoneme although the rate of subunit addition at the distal end is at least three times that at the proximal (Allen & Borisy *J. molec. Biol.* **90**, 381; 1974; Binder *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 1122; 1975). This directional polymerization is taken to reflect an intrinsic molecular polarity within the microtubule structure, that is, the asymmetric tubulin subunits are all oriented in the same way along the microtubule axis, an arrangement also inferred from detailed analyses of microtubule structure by electron microscopy and optical diffraction (Amos & Klug *J. Cell Sci.* **14**, 523; 1974).

Microtubule polarity has been a central concern in studies of mitosis ever since a suggestion made over a decade ago by McIntosh, Hepler and van Wie (*Nature* **224**, 659; 1969), that the polar functions of the mitotic spindle in mammalian cells might reflect an antiparallel relationship of microtubules originating from the polar centrosomes and the chromosomal kinetochores. The subsequent demonstration by many workers that both of these components would nucleate the assembly of neurotubulin in cell-free systems (McGill & Brinkley *J. Cell Biol.* **67**, 189; 1975; Snyder & McIntosh *J. Cell Biol.* **67**, 744; 1975; Telzer *et al. Proc. natn. Acad. Sci. U.S.A.* **72**, 4023; 1975) appeared, at least superficially, to be consistent with the McIntosh *et al.* model.

A more recent series of papers from Margolis and Wilson, however, has struck a discordant note. On the basis of an analysis of the exchange of [³H]GTP with microtubules assembled to steady state, these workers have proposed a novel mechanism of microtubule assembly whereby subunits are added to one end of the microtubule and removed at the other in a unidirectional (opposite end) assembly and disassembly process (*Cell* **13**, 1; 1978). The result is a continuous flow, or 'treadmilling' of subunits along the microtubule at a rate measured to be 0.69 µm h⁻¹. Extrapolation of these findings to

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*The Visual System of Fish, organized by M.B.A. Djamgoz (Imperial College, London) was held at Imperial College on 27 February, 1980.

the microtubules of the mitotic spindle has yielded a picture of spindle organization which differs radically from that outlined above (Margolis *et al.* *Nature* **272**, 450; 1978). Briefly, Margolis *et al.* envisage that microtubules assembled at the poles grow by the addition of subunits at the end distal to the organizing centre, while those assembled at the chromosomes are extended by the insertion of subunits proximal to the kinetochore. The result is a spindle in which the microtubules reveal a parallel orientation either side of the equator. Such an arrangement is not without appeal. The continuous insertion of subunits to both chromosomal and polar microtubules in the equatorial region and their subsequent removal at the poles might be expected to produce a constant poleward migration of material, a phenomenon often seen in the spindles of living cells. Although the measured rates of subunit treadmilling *in vitro* are generally lower than motions associated with living spindles, more recent work from the same laboratory has shown that the *in vitro* rate can be increased some 20-fold by the addition of 1 mM ATP to their assembly soup, giving a rate more consistent with that *in vivo* (Cell **18**, 673; 1979).

Unfortunately for such an elegant concept, the model was destined for a relatively short half-life, a situation by no means without precedent in the mitosis literature. Its demise was signalled by the appearance of two papers from the University of Wisconsin (Bergen & Borisy *J. Cell Biol.* **84**, 141; 1980; Bergen *et al.* *J. Cell Biol.* **84**, 151; 1980). Following an earlier suggestion from the same laboratory (Borisy *J. molec. Biol.* **124**, 565; 1978), these workers have undertaken a detailed analysis of the kinetics of polymerization and depolymerization of microtubules initiated *in vitro* by the centrosomes and kinetochores of Chinese hamster ovary cells. As a unique added refinement, Bergen and his colleagues have included in their analysis, *Chlamydomonas* flagellar axonemes as an unambiguous internal marker of directional microtubule growth. Thus it was possible to determine whether microtubules assembled onto the spindle organizers did so at a rate equivalent to that seen at either the proximal or distal ends of the flagellar standards alone, or alternatively at a rate which was a summation of the two reactions. The results were unequivocal. The rate of assembly into the mitotic organizers was identical to that at the distal end of the axoneme only, irrespective of whether conditions also supported the proximal extension of the internal standard. Equally important, a corresponding survey of the rate of depolymerization showed that this too was equivalent solely to the reaction taking place at the distal end of the added axonemes. Both findings conflict with the view of the spindle proposed by Margolis *et al.* in several important respects. Both

polar and chromosomal microtubules grow by addition of subunits distal to their site of initiation. Their arrangement in each half of the spindle must consequently be antiparallel. Finally, addition and removal of subunits takes place at the same, and not opposite, ends of the microtubule.

Intriguing as these assembly studies might be, there are encouraging signs that their days as the sole experimental handle on microtubule directionality are numbered. An exciting paper by Haimo, Telzer and Rosenbaum (*Proc. natn. Acad. Sci. U.S.A.* **76**, 5759; 1979) suggests that the long-awaited morphological marker of microtubule polarity might at last be at hand. The candidate is dynein, the ATPase protein responsible for the movement of cilia and flagella and seen as paired projections, or arms, connecting the 9+2 microtubules. Haimo and her colleagues have shown that axonemal dynein from *Chlamydomonas* added to microtubules during or subsequent to assembly binds periodically along the length of the microtubule, forming a complex reminiscent of the 'decoration' of

microfilaments by heavy meromyosin. The centre-to-centre spacing of the dynein projections is 24 nm, the same as its periodicity in the flagellum from which it derives. More important, when these reconstituted dynein arms were examined in the electron microscope they were characteristically tilted when viewed in longitudinal section and hooked when seen in transverse profile. Thus the arms can be used to define microtubule polarity in either orientation.

Although it is a long step from these *in vitro* studies to the *in situ* decoration of microtubules like those of the mitotic spindle, such an application might prove to be particularly appropriate. The anaphase movement of chromosomes in lysed mammalian cells has been shown to depend on the presence of ATP (Cande *et al.* *Proc. natn. Acad. Sci. U.S.A.* **71**, 573; 1978). Whether dynein is eventually shown to be the force-generating component of the spindle remains to be seen. What is clear is that this protein is going to have a central role in an exciting new era of microtubule research. □

The metal-semiconductor contact

from D.C. Northrop

METAL-semiconductor contacts (Schottky barriers) are commonplace in modern semiconductor electronics. Not only do they perform the active function of rectification in devices as widely different as microwave detectors and high current rectifiers, but they serve as ohmic contacts to virtually every semiconductor device that is made. Because of their evident importance such contacts continue to provoke widespread research interest on two quite separate fronts. One of these concerns the yield and reliability of semiconductor devices and microcircuits, where the metal-semiconductor contact and its associated metal wire bond are the most fallible part of the whole system. The other area of research, and the one that concerns us here, is the search for understanding of the physics of the contact; the relationship between its electrical properties and its structure.

Historically, the earliest attempt to provide a physical model for potential barrier formation was that commonly attributed to Schottky (Schottky *Naturwissenschaften* **26**, 843; 1938), but more correctly credited to Mott (Mott *Proc. Cambridge Phil. Soc.* **34**, 568; 1938) in the form in which it is usually stated. This model proposes that the electrostatic potential barrier opposing electron flow from the semiconductor to the metal is equal to the difference between the work

functions of the metal and the semiconductor making up the contact. This has an elegant simplicity about it and held sway for many years, partly because work functions are notoriously hard to measure experimentally, making the theory difficult to check. The first workers to provide adequate experimental results for metal contacts to clean silicon were Allen and Gobeli (*Phys. Rev.* **127**, 150; 1962) who found barrier heights that were essentially independent of the work function of the metal, apparently confounding the theory. Their results led to the acceptance of an idea proposed earlier by Bardeen (*Phys. Rev.* **71**, 717; 1947). He added to the Mott-Schottky model the concept of surface states; localised energy levels at the surface, whose electron occupancy had a controlling effect on the electric field in the semiconductor. This allowed a qualitative explanation of Allen and Gobeli's results, and also accounted for the great variability of other less carefully obtained experimental data.

Then followed the longest and most difficult phase of the work. Experimental techniques improved in every way; better vacuum systems, a greater number of very pure metals and semiconductors and more sensitive measuring techniques have all contributed to the search for these elusive surface states. Theoretical physicists have been at work too, and have played an important part in guiding experimental work. Particularly influential in this area have been Heine (*Phys. Rev.* **138A**, 169; 1965) and Inkson (*J. Phys. C*, **6**, 1359;

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1973), who pointed out that when metal and semiconductor are placed in intimate contact their surfaces will both be changed, particularly by the ability of electrons to penetrate from the metal into the surface of the semiconductor by tunnelling into the forbidden energy gap. This effect changes the charge distribution and therefore the electric field at the interface in a way that could explain qualitatively quite a large body of results. Gradually it has become clear, however, that quantitatively the effects predicted are not large enough to explain the number nor the spatial distribution of surface states. The results of Thanailakis (*J. Phys. C*, **8**, 655; 1975), are amongst the most important in establishing this point.

We come now to the latest suggestion, backed by extensive and impressive experimental data from Spicer and his group at Stanford University. The latest paper (Spicer *et al. Phys. Rev. Lett.* **44**, 420; 1980) spells out the mechanism they propose for barrier formation in a number of compound semiconductors. These are materials which exhibit no intrinsic surface states within the forbidden energy gap. That is, on the cleaved surfaces of these materials the Fermi Energy is found, by

photoelectric emission measurements, to be the same as that in the bulk, showing that there is no electric field in the semiconductor surface. The evaporation of metal, or the adsorption of oxygen, on these surfaces is found to change this situation and to give rise to surface states and, consequently, to electric fields which move the band edges relative to the Fermi Energy. The most important feature of their results is that the energy levels so produced are specific to the semiconductor and not to the material deposited. Spicer's explanation of this depends on the large heat of adsorption of materials on semiconductor surfaces, about 3 eV per atom according to some measurements. This is enough to produce lattice defects such as vacancies or more complex entities in and near the semiconductor surface, and it is these defects, Spicer claims, which control the potential barriers. It is perhaps early to say that the full picture has now been revealed, but it is clear that an important new step has been taken. It will lead to a better appreciation of what is possible in the production of metal-semiconductor contacts with tailored properties as well as improving our understanding of interface physics. □

virus equivalent of SV5-induced HN antibodies), and would provide additional antigenic stimulation resulting in an anamnestic response to the H antigens to which the vaccine provided the primary response. In these conditions, immune complexes composed of the antigen-producing syncytium and H antibodies could form, activate the complement system and result in inflammation and tissue necrosis. Alternatively, antibody-mediated cytotoxicity might cause the observed inflammation without complement activation. Either process could account for the immunopathological reactions that have been observed in some people given RS or measles vaccines.

In addition to providing an insight into the possible mechanisms involved in the complications associated with paramyxovirus vaccination, the work of Merz *et al.*, together with earlier work by Waldman and Ganguly (*J. infect. Dis.* **130**, 419; 1979) and McIntosh *et al.* (*J. infect. Dis.* **138**, 24; 1978), among others, suggests an approach to paramyxovirus vaccination that may be both safe and effective. Merz *et al.* show that pure F glycoprotein would be the ideal immunogen for a paramyxovirus vaccine and the earlier studies suggest that the most effective way of administering this immunogen would be directly into the lung as an aerosol. The advantage of such local immunization is that it would stimulate secretory IgA, which does not activate the complement system or antibody-mediated cytotoxicity, and would thus avoid the immunopathological consequences resulting from stimulation of IgG antibodies. The disadvantages of the suggested approach are the difficulty of developing high IgA titres and the short duration of immunity. However these problems are not insurmountable. Morein *et al.* (*Nature* **276**, 716; 1978), for example, were able to prevent a lethal infection of mice with Semliki Forest virus by vaccinating the mice with a micellar aggregate of the virus spike protein. Interestingly, they found that the monomer form of the protein solubilized with detergent was much less effective than the aggregated protein. Those results suggest that protective and lasting immunity to paramyxoviruses might be stimulated by administering the F glycoprotein as an aggregate rather than in the solubilized form. Alternatively, the F glycoprotein could be administered together with an immunoadjuvant, such as the purified active component of Freund's adjuvant, N-acetyl-muramyl dipeptide (Azuma *et al. Infect. Immun.* **14**, 18; 1976; Chedid *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 2472; 1976), which does not produce the abscesses associated with the complete Freund's adjuvant. Using either approach, it might be possible to introduce pure F glycoprotein into the lung as an aerosol and stimulate immunity without adverse side effects. If so, it would be an important step towards controlling respiratory infections.

Vaccination against paramyxoviruses

from A.J. McClelland

PARAMYXOVIRUSES are now known to be a major cause of respiratory illnesses but, despite the importance of these diseases, little progress has been made in their control. Live virus vaccines are available for only two paramyxovirus diseases (measles and mumps) and failures of these vaccines resulting in severe complications, such as modified and atypical measles, have been reported (Chatterji & Marikad *J. Am. Med. Assoc.* **238**, 2635; 1977). Inactivated virus vaccines against the measles virus and other paramyxoviruses, such as the respiratory syncytial (RS) virus and the parainfluenza viruses, have had limited success and have sometimes induced severe forms of the illnesses they are supposed to prevent (Kim *et al. Am. J. Epidemiol.* **39**, 422; 1969; Welliver *et al. Arch. Int. Med.* **137**, 39; 1977). The unusual complications that sometimes occur after paramyxovirus vaccination have been attributed to an aberrant immune response, but it is not known how or why such a response occurs.

New light has recently been shed on the problems associated with paramyxovirus vaccination by Merz *et al.* (*J. exp. Med.* **151**, 275; 1980) who have investigated the infectious spread of one paramyxovirus, SV5, using monospecific antibodies to the two SV5 surface glycoproteins. These

authors report that SV5 can be disseminated in two ways: (1) release of infectious virus from infected cells and (2) cell fusion, in which no infectious virus need be released. They found that antibodies to the viral haemagglutinating and neuraminidase (HN) glycoprotein prevent the spread of infection by the release of virus particles, but do not prevent the spread of infection by cell fusion, whereas antibodies to the viral fusion (F) glycoprotein prevent the spread of infection by both cell fusion and release of infectious virus. The results obtained by Merz *et al.* emphasize the importance of the F glycoprotein in the dissemination of SV5.

Their findings, together with the earlier observation that vaccination elicits a weak F antibody response, or fails to stimulate F antibody production altogether (Norrby & Gollmar *Infect. Immun.* **11**, 231; 1975), suggest a possible explanation for the severe complications that sometimes result from paramyxovirus vaccination. In a person vaccinated against measles, for example, exposure to the measles virus could result in a disseminated infection because of the lack of F antibodies and the ability of the virus to spread from cell to cell by cell fusion. As the infection spreads, syncytium formation would take place and the antigen load would increase. Released virus particles would be neutralized by the vaccine-induced H antibodies (the measles

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REVIEW ARTICLE

Fluorescently labelled molecules as probes of the structure and function of living cells

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A new approach to cell biology has been created by combining the techniques of micromanipulation of cells, fluorescence spectroscopy and low level light detection. These methods are now being used to study the spatial and temporal distribution, interaction and activity of specific molecules in living cells.

CELL functions such as locomotion, cell recognition, endocytosis, exocytosis, and cell division involve highly dynamic and transitory molecular interactions. Early cell physiologists studied these functions by probing living cells and developed sophisticated techniques for micromanipulation and microinjection¹⁻⁴. A new approach bridging the gaps between cell physiology, biochemistry and ultrastructure is required to define the molecular mechanisms of complex cell functions. Such an approach would require a combination and coordination of (1) techniques for manipulating living cells, (2) techniques which provide signals from specific molecules with high sensitivity, and (3) techniques which detect weak signals from living cells. Recently, the methods of micromanipulation of cells¹⁻⁵, fluorescence spectroscopy⁶⁻⁸, and low-level light detection⁹⁻¹³ have been combined to provide such a new approach to cell biology. This new concept involves labelling purified molecules covalently with fluorescent probes, and incorporating the fluorescent conjugates into or onto living cells. Cells with associated fluorescent conjugates are then either viewed with an image intensifier coupled to a microscope or are investigated with a microspectrofluorometer. Therefore, the high sensitivity ($\sim 10^5$ – 10^6 molecules can be detected¹⁴), and versatility⁶⁻⁸ of fluorescence techniques can be fully utilised in living cells to yield information at the molecular level.

This review is limited to applications of this approach in which purified molecules are labelled, administered and then studied at the light microscopical level. The technical aspects will be discussed in detail since this new approach requires careful application and interpretation. In addition, three categories of fluorescent conjugates will be discussed: (1) nonperturbing indicators of physiological processes, (2) biologically active agents and (3) functional cellular components (molecular cytochemistry)¹⁵. Some of the studies referenced could be included in more than one of these categories.

Experimental methods

The application of fluorescent conjugates to studies in single living cells demands the use of highly fluorescent fluorophores which absorb in the visible spectrum, so that adequate signals are obtained while minimising radiation damage and interference from autofluorescence¹⁶. Furthermore, the conjugates must be associated by stable covalent bonds and be devoid of non-covalently bound fluorophores. Recent advances in the preparation of fluorescent reagents have provided several compounds suitable for applications *in vivo*. Fluorochromes such as fluorescein¹⁵⁻¹⁷, eosin¹⁸, 7-chloro-4-nitrobenzo-2-oxadiazole (NBD)¹⁹, and a series of long-wavelength rhodamine dyes^{15,20-21} have been used successfully with living cells. In addition, various reactive derivatives of these fluorophores such as iodoacetamide¹⁵, isothiocyanate²⁰ and sulphonyl chloride¹³ can be obtained commercially. Many of the long-wavelength

fluorophores used primarily by neurophysiologists should be useful when reactive derivatives become available²².

Classical microinjection techniques are still the most direct methods for incorporating exogenous components into living cells^{1,23}. Several different microinjection systems, using both hydraulic pressure^{4,5,17} and compressed air^{13,24,25}, have been described in detail. Cells ranging in size from giant protozoans ($\sim 600 \mu\text{m}$) to human fibroblasts ($\sim 15 \mu\text{m}$) have been successfully microinjected. These techniques are time consuming and the numbers of cells which can be studied are limited. However, they have the advantages that only a very small volume of material is required for each experiment; components as large as

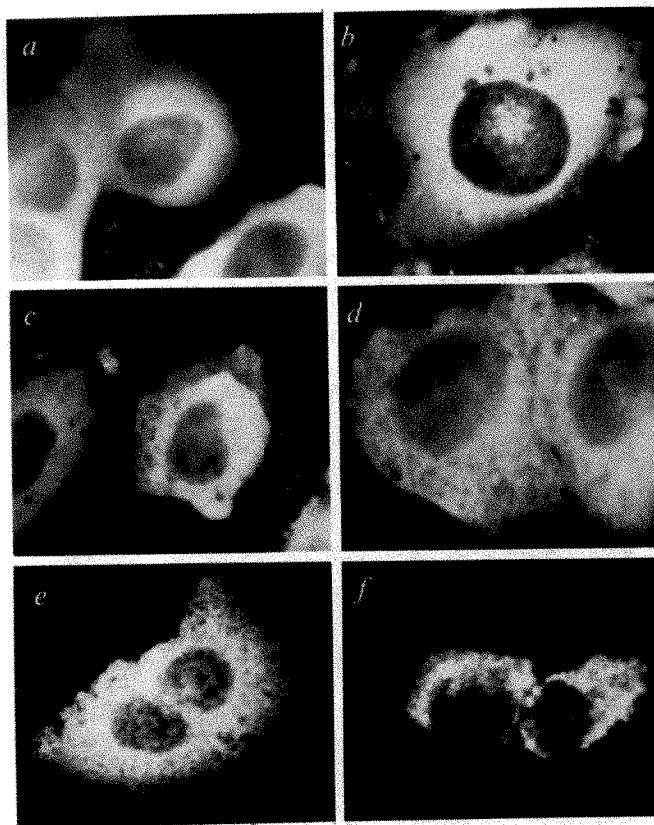


Fig. 1 Autophagy studied by injecting highly concentrated tetramethylrhodamine isothiocyanate labelled bovine serum albumin into HeLa cells. These fluorescence micrographs were taken of injected cells *a*, immediately; *b*, 30 min; *c*, 1 h; *d*, 90 min; *e*, 6 h thereafter. The labelled proteins were taken up into vesicles which ultimately fused with lysosomes (*f*). (From Stacey, and Allfrey⁴⁵.)

organelles can be microinjected⁴, mild solution conditions can be used to deliver the fluorescent conjugates^{13,15,17,20}, and injection volumes can be controlled.

During the past few years, new delivery methods using fusion techniques have been developed. The molecules are trapped inside carriers such as red blood cell ghosts²⁶ and liposomes^{27,28} which are subsequently fused with target cells. While these methods allow the 'ultramicroinjection' of large populations of very small cells there are also several limitations: (1) relatively large volumes of fluorescent conjugates are required during the loading process, (2) the conjugates could become exposed to detergents, elevated temperatures, or organic solvents, (3) the proper entrapment and fusion steps must be carefully verified, and (4) the biological effects of the carriers must be controlled. Recently, fibroblasts have been successfully loaded with fluorescently labelled antibodies by red blood cell fusion techniques²⁹. Continued improvements in fusion technology should make these approaches more generally useful in the future.

Cell perfusion techniques have been used to deliver small ions and molecules into plant cells³⁰. A membrane permeation technique has now been reported which permits the incorporation of small molecules into living animal tissue culture cells³¹. The cells are permeabilised by short treatments with lysolecithin which make the cells leaky to exogenous molecules in the medium for a short period of time. This technique has advantages similar to those of fusion methods, yet does not require the use of carriers. Unfortunately, the application is limited to only very small molecules (molecular weight below 10,000) and to cells grown in monolayer culture. In addition, the cells remain viable for only a short period.

Recent developments in image intensification techniques have provided a more sensitive way of recording fluorescent images than classical photographic methods¹⁰. The use of standard photographic procedures requires long exposure times and intense illumination. Therefore, fluorescence photobleaching could be extensive, cell damage is possible, and dynamic processes are difficult or impossible to record. In contrast, TV image intensifiers coupled to fluorescence microscopes and video tape recorders allow the continuous recording of weakly fluorescent images in real time or time lapse without significant losses in resolution¹⁰. Some TV cameras also provide digital output for quantitative image analyses. When these intensifiers are coupled to optical prisms and multichannel analysers, they can also provide rapid microspectrofluorometric measurements^{10,32}.

Microspectrofluorometers have been developed for quantitative measurements of many fluorescence parameters with very low light intensities^{33,34}. In particular, photomultipliers

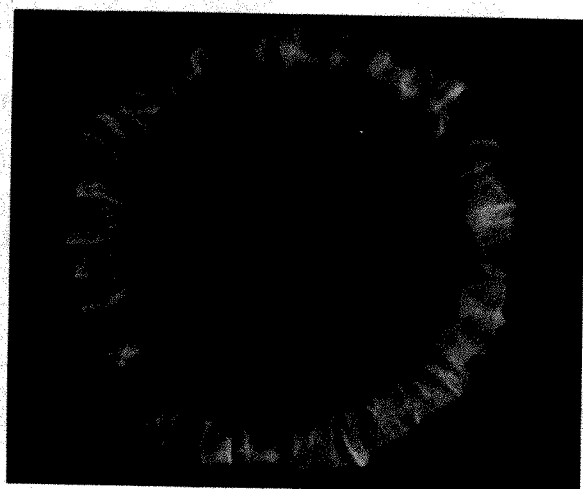


Fig. 2 Fluorescence photograph of a live chick embryo fibroblast that is lysolecithin permeabilised and labelled with NBD-phalloidin¹⁹. Most of the labelled actin in this spreading cell is located peripherally in the area of membrane ruffling. Some longitudinal stress fibres are also evident. (Micrograph courtesy of L. Barak.)

PURE ACTIN

Label with 5-iodoacetamidofluorescein

SELECT FUNCTIONAL AND OPTIMALLY LABELLED ACTIN

- (A) Polymerisation-depolymerisation
- (B) DEAE chromatography

CHARACTERISE LABELLED ACTIN

- (A) Dye/protein
- (B) Site of labelling
- (C) Polymerisability
- (D) Activation of myosin Mg^{2+} ATPase

DETERMINE FUNCTIONAL ACTIVITY *IN VITRO*

- (A) Single cell models
- (B) Bulk extracts

INCORPORATE LABELLED ACTIN INTO LIVING CELLS BY MICROINJECTION

Limit: 10% of endogenous actin concentration

DETERMINE FUNCTIONAL ACTIVITY *IN VIVO*

CORRELATE DISTRIBUTION OF ACTIN FLUORESCENCE WITH OVALBUMIN CONTROL

Fig. 3 Flow diagram of the steps involved in molecular cytochemistry. Actin is described as an example.

interfaced with single photon counting devices have provided instruments of high sensitivity³⁵. These systems in combination with computer-controlled spectral scanning and data processing form the core of several sophisticated microspectrofluorometers which are capable of performing corrected spectral measurements on single living cells³⁵⁻³⁸. The use of lasers for excitation has also increased the versatility of microspectrofluorometers, because the state of polarisation, spot size, duration and intensity of the exciting light pulse can be controlled over large ranges.

Recently, fluorescence photobleaching recovery techniques have been introduced to measure the mobility of surface associated fluorophores³⁹⁻⁴². These methods utilise a pulsed high-intensity laser microbeam to bleach a small area or volume of fluorescence, and the recovery of fluorescence is monitored with attenuated laser excitation. These measurements can yield diffusion constants of the mobile fraction, the percentage of fluorescent conjugates that are mobile, and information on the bulk directional flow of the conjugates. However, caution must be exercised in controlling the possible biological effects of photobleaching⁴³, and consideration must be given to the validity of the assumption that cell surfaces are flat and smooth. In addition, care must be taken to ensure that the membrane or surface markers are not internalised before making measurements. These potential problems have recently been addressed critically by Elson and Yguerabide⁴⁰.

Physical and chemical considerations

There are several possible physical and chemical problems which could give rise to experimental artefacts, and investigations using this new approach must be judged in part by the manner in which potential problems are considered. Factors which could affect the fluorescence intensity measured for the microscope include: (1) local accessible volume for the conjugate in the cell, (2) local environment of the conjugate, (3) optical properties of the microscope, and (4) local concentration of the conjugate.

Consideration of the local accessible volume is important for both cytoplasmic and membrane-associated components. For cytoplasmic components, the local accessible volume is controlled not only by the actual thickness of the cell, but also by the distribution of organelles which exclude the fluorescent conjugates. For membrane-associated components, the accessible volume is affected dramatically by the presence of membrane folds and microvillar structures. This problem can be

identified and controlled by using a second fluorescent conjugate which would distribute uniformly within the accessible volume. Labelled bovine serum albumin, ovalbumin, or denatured proteins have been used in controls for cytoplasmic components^{13,15,17,20} and fluorescent lipid probes⁴¹ can be used in studies on membrane-associated factors. It is imperative that co-incorporation of the experimental conjugate and the control conjugate labelled with a second probe be accomplished in the same cell when the cell shape is irregular¹³. Adequate comparisons cannot be performed in separate cells when they have irregular geometries. However, separate cells can be used when the cell geometry is reasonably constant and simple¹⁷.

The local environment around the fluorescent conjugate could also affect the fluorescence intensity by altering the quantum yield or fluorescence spectral properties. Local variations in ionic parameters such as pH and ionic strength as well as binding of other molecules to the conjugates could change the fluorescence parameters^{6,7}. The sensitivities of fluorophores can either be utilised in characterising microenvironments or they must be controlled when other parameters are under investigation. Controls for the ionic environments can be performed by comparing the fluorescence of the experimental conjugates with the fluorescence of separate cells containing nonspecific molecules (such as ovalbumin) labelled with the same fluorophore¹⁷, while controls for the effect of specific molecular interactions require the extrapolation of data from solution spectroscopic studies¹⁶.

Knowledge of the optical properties of the microscope is important for interpreting fluorescence images and quantitating local fluorescence properties. The characterisation of the fluorescence image *in vivo* depends on the depth of focus of the microscope. A large depth of focus in relation to cell thickness

optimises the formation of an in focus image of the whole cell, while a small depth of focus yields an optical section of the cell. The latter condition would require several changes in focus to reconstruct the three-dimensional image of the cell. Quantitative measurements of fluorescence intensity are further affected by the extent of selecting light from specific planes of the specimen. This latter problem has been solved by using a combination of laser illumination and diaphragms placed in the image plane³⁹.

The local concentration of the fluorescent conjugates also affects the fluorescence intensity measured or visualised in different parts of cells. The distribution of the conjugates can be determined by applying a combination of the controls used for accessible volume and environmental sensitivity.

Nonperturbing indicators of physiological processes

Nonperturbing fluorescent conjugates can be used as indicators of mobility on, within, or between cells. Fluorescence indicators of normal cell-surface mobility have been studied for many years. In an early study Jeon and Bell⁴⁴ used fluorescently labelled antibodies to the cell surface of *Amoeba proteus* and a basic protein derived from a papain preparation to label the cell surface. Results based on a double labelling technique suggested that part of the cell surface could move independently of the lipid portion of the membrane.

In an elegant study, rhodamine-labelled peptides of different sizes have been microinjected into living cells coupled by gap junctions to probe the permeability and exclusion limit of the gap junction channels^{45,46}. Molecules of molecular weight up to 1,200 pass through the channels of *Chironomus* salivary gland cells which indicates that the channels are ~1.0–1.4 nm in diameter. The selectivities of the various channels have been further characterised by varying the total charge of the labelled peptides⁴⁵. For example, several mammalian cell channels can discriminate between 1–3 negative charges on the peptide backbones. The larger electronegativity inhibits passage. In addition, the permeability of multiple components has been directly compared by using mixtures of conjugates prepared with different probes^{45,46}. A control for peptide degradation has also been reported.

Stacey and Allfrey used a similar technique and microinjected a wide variety of rhodamine conjugated proteins into living HeLa cells⁴⁷ to study the process of autophagy (Fig. 1). The segregation of microinjected proteins into autophagic vacuoles exhibited a high degree of selectivity with higher molecular weight proteins turning over faster than low molecular weight proteins. One protein (haemoglobin) never became autophagocytosed. The results with fluorescently conjugated proteins were verified with immunofluorescent and autoradiographic techniques.

More sophisticated applications of nonperturbing fluorescent conjugates involve the use of environmentally sensitive fluorophores and spectral analyses to probe intracellular environments. Fluorescein-labelled ovalbumin has been used to measure the cytoplasmic pH of single cells⁴⁸ based on the observation that the excitation spectra of fluorescein is highly pH dependent⁴⁹. The pH is measured by determining the ratio of fluorescence emission intensity when the cells are excited at two different wavelengths. The effects of local pathlength are normalised since the ratio of intensities is determined. This fluorescence technique of measuring pH is less perturbative and permits better spatial resolution than standard microelectrode methods.

When applying fluorescent conjugates as indicators, the biological effects of the conjugates and the experimental procedures should be carefully examined to make sure that they are actually nonperturbing. The possible degradation of the conjugates inside the cells should also be considered, especially when the conjugates are used as size indicators^{46,47}. This latter problem could be checked more critically in the future by isolating the labelled proteins from the cells after incorporation

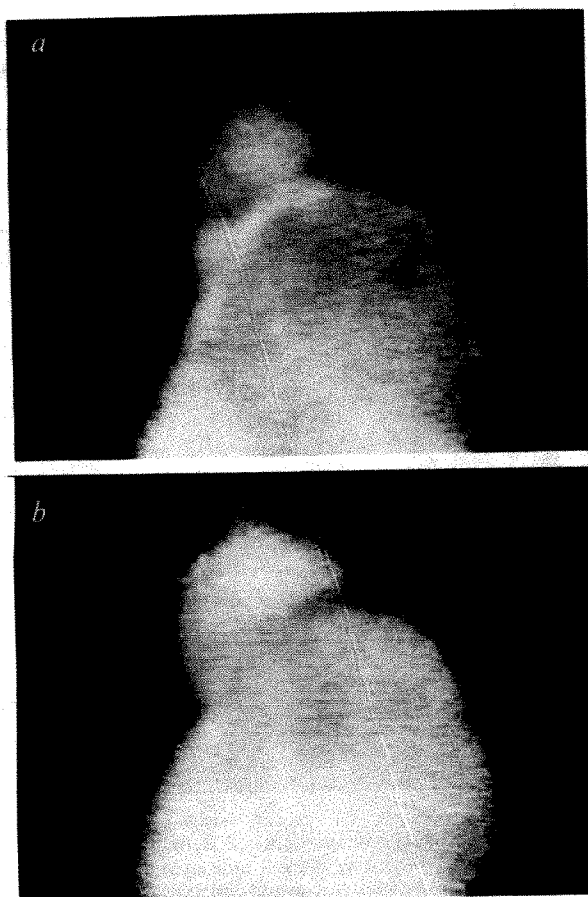


Fig. 4 Fluorescence of 5-iodoacetamidofluorescein-labelled actin (a) and lissamine rhodamine B sulphanyl chloride labelled ovalbumin (b) in the same specimen of *C. carolinensis*. Actin specific fibrils can be detected in the plasmagel sheets at the tips of advancing pseudopods (a). (From Taylor *et al.*¹³.)

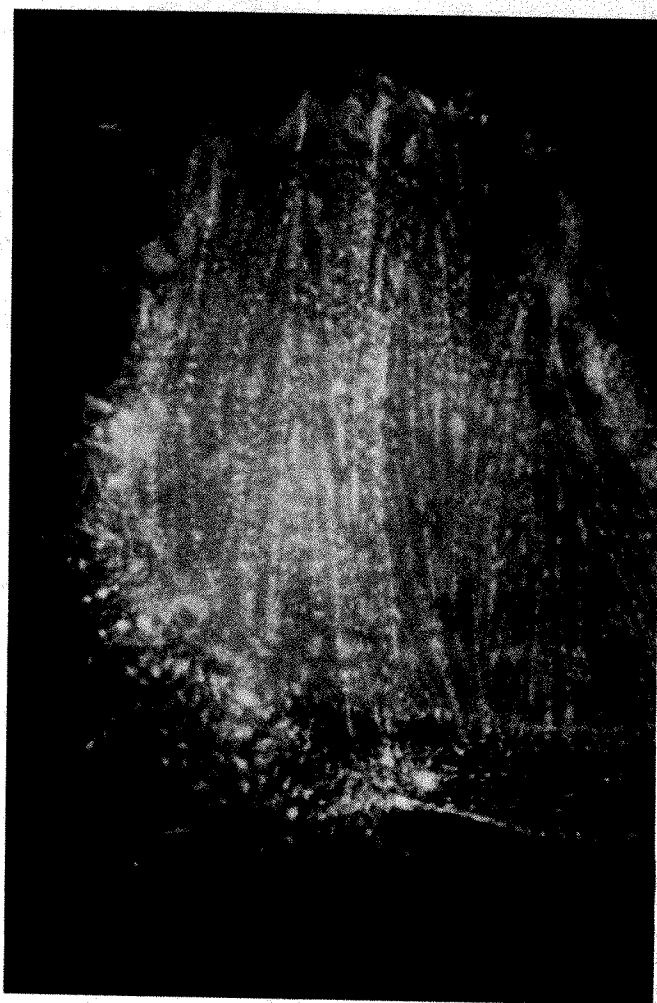


Fig. 5 Tetramethylrhodamine isothiocyanate labelled α -actinin incorporated into a living gerbil fibroblast cell. (From Feramisco²⁰.)

by fusion techniques. Furthermore, when spectral characteristics are used as indicators of local environments such as pH, the possible effects of other environmental factors must be ruled out. In all cases, calibration must be performed *in situ*⁴⁸.

Biologically active agents

Biologically active agents which can stimulate, alter or block cell functions include lectins, antibodies, drugs and toxins. These agents are used in probing cell structures and functions. Many of these agents can be fluorescently labelled, permitting detailed investigations on the distribution and mobility of binding sites. The relationship between distribution and biological effects of the labelled agents can also be studied.

The plant lectin, concanavalin A (Con A), has been used as a model ligand in investigations on receptor-ligand complexes⁵⁰⁻⁵⁷. Fluorescently labelled Con A has facilitated studies on the mobility of Con A receptors, the ultimate fate of the ligand and the relationship between receptor mobility and Con A stimulated cellular events⁵¹⁻⁵⁷. It must be emphasised that Con A has many complex effects on cells⁵⁷ and it is difficult to define the complete molecular sequences of events. Similar but more specific experiments have also been performed with antibodies prepared against various cell-surface receptors⁵⁶⁻⁵⁸. These cell-surface associated fluorescent conjugates have been used to measure the mobility of membrane components using several different approaches. The simplest method is the observation of large cells such as muscle fibres⁵⁹ in the fluorescence microscope. Small labelled spots on the cells can be detected when spread over relatively large distances. A second approach involves fusing cells containing different surface fluorescent

conjugates and following the redistribution of the labelled conjugates in the microscope⁶⁰. Poo and colleagues⁶¹ have also studied the accumulation of labelled surface receptors in a uniform electric field applied across the surfaces of cells. Finally, several investigators have used the photobleaching techniques discussed above.

Some drugs can also be fluorescently labelled while maintaining activity. The purpose of labelling the drugs is to study the correlation between the cellular effects and the localisation within the cell. Examples are the tubulin-binding drug colchicine⁶², and the actin-binding cyclic peptides phalloidin¹⁹ and phalloidin⁶³. At the proper concentration, colchicine can cause microtubule depolymerisation and phalloidin and phalloidin can cause actin polymerisation and stabilisation (see ref. 64 for references). Fluorescent conjugates of these drugs have been used to localise pools of tubulin⁶² and F-actin^{19,63} respectively in fixed or extracted cells (Fig. 2). However, it is not yet clear whether these drugs have simply identified pools of tubulin and F-actin or have also altered the normal organisation of the target proteins.

Functional cellular components (molecular cytochemistry)

Molecular cytochemistry¹⁵ has been defined as the re-incorporation of functional cellular components into or onto living cells following purification, fluorescent labelling and assaying function *in vitro*. The experimental protocol is shown in Fig. 3 using actin as a model. The feasibility of molecular cytochemistry has been initially demonstrated using actin labelled with a non-destructive probe, 5-iodoacetamido-fluorescein (IAF). The labelled actin has been proven to be functional in the purified form, in cell-free extracts, and in single-cell models^{13,15-17}. Microinjection of this fluorescent



Fig. 6 Saltatory motion of fluorescent vesicles in 3T3 cells 24 h after incubation with rhodamine-labelled α_2 -macroglobulin. A time lapse video tape recording at a 9:1 time lapse ratio was made of a cell with vigorous intracytoplasmic vesicle saltatory motion. The numbers in the lower right corner of each single frame image represent the real time in seconds after the beginning of the sequence. A phase image is presented at zero time followed by fluorescence images. The asterisk represents an arbitrary non-moving reference point. The inset summarises the motion of this single fluorescent vesicle. (From Willingham and Pastan¹².)

actin analogue into living cells has provided a direct way of visualising actin-containing structures and following the changes in actin distribution during cellular processes such as fertilisation of eggs¹⁷, cytokinesis¹⁷, amoeboid movement^{13,15}, pinocytosis¹³, and Con A capping¹³. Extensive controls *in vitro* and *in vivo* have made the interpretation of the fluorescent images possible^{13,15-17} (Fig. 4). This approach has recently been applied to other cells^{20,21} and other contractile proteins²⁰ (Fig. 5).

The use of fluorescent conjugates specific for receptors in the cell surface has permitted the characterisation of the kinetics of the distribution and the ultimate fate of many ligands. These investigations have included studies on α_2 -macroglobulin^{12,65,66}, epidermal growth factor⁶⁶⁻⁶⁸, insulin⁶⁶⁻⁶⁹, a chemotactic peptide⁷⁰, low-density lipoproteins⁷¹, and acetylcholine receptors⁷¹⁻⁷⁴. All of these fluorescent ligands initially label the cell surface uniformly, but at least some of the ligand-receptor complexes aggregate into patches within a few minutes. Most of the fluorescent conjugates eventually become internalised^{69,75}. The role of internalisation has not yet been defined in detail. The redistribution of some of these ligand-receptor complexes in the plane of the membrane has been quantitated using the fluorescence photobleaching techniques.

Proper use of molecular cytochemistry demands the application of various biological controls in addition to the analysis of physical considerations discussed above. The functional activity of the conjugates must be carefully demonstrated. It requires that: (1) the labelling reaction does not abolish the normal biochemical activity of the substrate, (2) the conjugates have access to the intracellular domains where the function is performed, and (3) the conjugates are not rapidly degraded *in vivo*. The biochemical activities can be readily tested using *in vitro* assays, cell-free extracts or lysed cell models^{13,16}. The use of site-specific probes would provide functionally homogeneous conjugates and would yield unequivocal results with *in vitro* assays¹⁶. The studies with labelled actin have yielded the most definitive biological controls^{13,15-17} to date.

The function inside living cells is much more difficult to assay. For IAF-labelled actin mentioned above, well characterised cellular responses such as cortical wound healing, can be used to test the functionality *in situ*¹³. Furthermore, a comparison of the fluorescent images of biologically active conjugates with non-functional conjugate controls would also indicate the formation of structures related to biological functions^{13,15,17}. Note that the absence of fluorescence from a cellular domain, such as the nucleus^{13,17}, does not necessarily imply that the endogenous component is not present. Negative results could be due to physical exclusion of the conjugate, slow turnover time for the incorporation into specific structures, as well as the absence of a cellular component from a particular region. Therefore, negative results must also be interpreted with caution.

Future prospects

This new approach to cell biology is likely to develop extensively both technically and in its range of biological applications. At the technical level, improvements in the hardware and software applied to intensified image recording and spectral analysis should permit sophisticated manipulations of the experimental data. Measurements of fluorescence polarisation^{14,76,77}, resonance energy transfer^{60,78-80} and fluorescence lifetime could be performed and factors such as accessible volume could be corrected automatically. Detailed molecular information including microviscosity, local polarity, rotational freedom and formation of supramolecular structures could be determined.

The application of this new technique is expected to extend to many different areas of cell biology, through the use of fluorescent conjugates of nonperturbing indicators, biologically active agents and functional cellular components. Components binding specific ions or ligands could be labelled with environmentally sensitive probes and used as indicators of intracellular environments. One such possibility would be the calcium binding protein calmodulin⁸¹. Some drug studies previously relying on radioactive derivatives could be performed with fluorescent

conjugates which would allow the direct observation of uptake, distribution and turnover. Furthermore, the new techniques of blocking cell functions with specific antibodies^{82,83} or modified cellular components⁸⁴ can be combined with the present approaches yielding a more powerful technique. The most dramatic advances will probably be seen in the area of molecular cytochemistry using functional conjugates of proteins, lipids, nucleic acids, carbohydrates and even whole organelles. Careful use of molecular cytochemistry is expected to bring new insights into areas as diverse as cell motility, virus-cell interactions, nuclear-cytoplasmic interactants, cell-cell interactions in tissues, axonal transport, gene expression, and assembly of organelles at both morphological and molecular levels. A direct connection between cell physiology, biochemistry and ultrastructure would then become a reality.

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ARTICLES

Variations in the thermal emission of Seyfert galaxies

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Accurate monitoring of five Seyfert galaxies demonstrates that they are all variable in the IR region. These fluctuations probably arise when variations in the nonthermal UV sources change the rate of heating of interstellar dust within the nuclei of these galaxies, as demonstrated quantitatively for III Zw 2.

AS soon as the large IR excesses of Seyfert galaxies were discovered, it was recognised that any variability of these excesses could be a vital clue to their nature, in particular as to whether they had a thermal or a nonthermal origin. Despite relatively large measurement uncertainties, early programmes produced evidence that variations do occur^{1–4}. It followed then that greater accuracy would be required to study the variations in any detail, as well as to convince sceptics of the reality of the changes observed. The large change reported recently for 3C120 in the IR⁵ seems to be exceptional, and any general monitoring programme should consider variations of 10–20%.

Recent advances in near IR detector technology make possible routine differential photometry between the brighter Seyfert galaxies and selected nearby stars with an accuracy of 1–2%. This article reports such photometry for III Zw 2, NGC1275, 3C120, NGC4151 and NGC 5548, galaxies selected because of their high degree of variability in the visible. During our observations, all five galaxies varied by many times the measurement errors. Comparison of coordinated IR and visible observations shows that the variations in the two spectral regions are not simultaneous. The behaviour of these galaxies indicates that much of the UV emission of their nuclei is absorbed by interstellar dust and is re-radiated in the IR.

New observations

Our photometry is listed in Tables 1 and 2. Most of the IR measurements were obtained on the Catalina 1.54-m (61-in) telescope, with a field of view 8.5 arc s in diameter and relative to reference areas 10 arc s to the east and west of the nucleus of the galaxy. A few measurements were made on the Steward 2.24-m (90-in), where a beam of 7.8 arc s was used with reference areas 10 arc s to the north and south of the nucleus. No systematic differences are apparent between measurements with the two telescopes.

Two photometers were used, one with a liquid nitrogen cooled InSb detector and one with a liquid helium cooled detector of the same type. Although the filters defining the spectral passbands were identical for the photometers, the lower operating temperature with the second instrument produced slight shifts in filter characteristics. The effects of these shifts

were calibrated and removed from the data by comparing measurements with both photometers in August 1978.

The tabulated errors for the IR photometry reflect only the combined scatter of statistical uncertainties and of a series of comparisons with the local standards on that night. Typically, three or four comparisons were made in each colour. The measurements have been converted to fluxes by tying the local standards in with the network established by Johnson *et al.*⁶ and applying the calibration at $J(1.25 \mu\text{m})$ of 0.0 mag = 1,770 Jy, at $H(1.6 \mu\text{m})$ 0.0 mag = 1,080 Jy, at $K(2.2 \mu\text{m})$ 0.0 mag = 680 Jy, and at $L(3.5 \mu\text{m})$, 0.0 mag = 300 Jy. We believe that the local standards are tied into the primary system with an accuracy of at least 3% (6%, for NGC5548). The absolute calibration is also subject to an uncertainty of 5–10%. Fortunately, for virtually all of our analysis only the uncertainties in the comparisons with the local standards are important.

Photoelectric measurements were obtained with a Johnson *UBVRI* photometer on the Mt Lemmon 1-m (40-in) telescope. The comparison sequences listed by Lyutyi⁷ were used except for III Zw 2, where local standards were determined as part of this programme. As with the IR observations, the tabulated errors refer only to the comparisons with the local standards. The absolute calibration is described by Johnson⁸. The measurements of III Zw 2 on 11 December 1979 were provided by Wieslaw Wisniewski.

Nature of IR variations

Tables 1 and 2 also include an average over the measurements in the J , H , K and L spectral bands for each date, normalised to 1.00 for an arbitrary date and weighted by the tabulated errors. This parameter, plotted in Fig. 1, is convenient for showing the extent of overall variability, although in some cases we have found a strong spectral dependence to the changes (note III Zw 2, discussed below). The same parameter is shown for previous measurements⁵, in the cases of III Zw 2 and 3C120, the parameter is normalised to the data of August 1978, reported in this earlier publication. As is clear from Fig. 1, all five of the galaxies show IR variability at a high degree of confidence.

The five galaxies were selected for this programme on the basis of their large amplitude optical variability. In other

Table 1 Coordinated optical and IR photometry of Seyfert galaxies

Source	Date (UT)	U	B	V	R	I	J	H	K	L	(IR)	$\delta(\text{IR})$
III ZW 2	8.27.78	3.9*	4.65†	5.63†	7.4*	7.0*	9.1†	12.2†	22.1		0.99	0.02
	1.5.79		4.88				10.1	13.3	23.2		1.05	0.02
	9.20.79	2.74†	3.13	4.17	6.0‡	5.4‡	8.8†	14.0	27.1		1.16	0.02
	12.3.79	3.30†	3.73	4.48†		7.5‡	8.6	13.7	26.3		1.13	0.02
NGC1275	8.27.78	7.4†	15.1	23.7	35*	53†	55	69	93		1.00	—
	11.19.78	6.0*	13.4	21.2	32*	43†	37	50	67		0.70	0.01
	9.20.79	6.7†	12.9	20.9†			40.5	51	67†		0.73	0.01
	12.5.79		12.5	20.5	34*	45†	35	46	63		0.65	0.01
3C120	8.27.78	1.40*	1.85†	3.80†			10.8†	15.0	23.6		1.07	0.02
	11.19.78		2.35†	4.0*			10.7	14.2	24.1		1.07	0.02
	9.20.79		2.2†	4.4*			10.0†	17.1	28.3		1.18	0.02
NGC4151	4.3.78	39†	48	65	107	122	127	176	227	371	1.00	—
	3.7.79	16.1†	29.6	47	87	97	86	116†	162		0.68	0.01
	5.11.79	31.2	44.8	63	105	118			152		0.67	0.02
NGC5548	4.3.78	11.4†	14.3	21.0	31.8	36.1	35.0	42.2	57.1	90	1.00	—
	3.7.79	5.8†	8.3†	13.4			24.2	31.4	42.5		0.72	0.02

0 < errors < 2% unless otherwise indicated; the error in (IR) (the normalised IR brightness) is indicated as $\delta(\text{IR})$ and is based on the inverse square weighting of the changes in the IR bands.

* 4% < errors ≤ 6%. † 2% < errors ≤ 4%. ‡ 6% < errors ≤ 10%. Fluxes in mJy = $10^{-29} \text{ W m}^{-2} \text{ Hz}^{-1}$. 23 arc s aperture for *UBVRI*, 8.5 arc s aperture for *JHKL*.

respects, they represent a very broad range of spectral properties and luminosities. As they all varied, we believe that virtually all Seyferts that vary in the visible also vary in the IR.

The simplest explanation for variations in both spectral regions would be that a single, nonthermal source dominates the output of these galaxies from the visible through the IR. However, the variations are not simultaneous between the visible and IR, and in some cases they even occur in the opposite sense in the two spectral regions. This behaviour differs dramatically from that of nonthermal sources such as BL Lac type objects⁹. We therefore will consider models in which different sources dominate the IR and optical spectra.

For these models, the spectra of the nuclei of the galaxies can be divided into four components: (1) a rapidly fluctuating, presumably nonthermal, source that dominates the UV continuum; (2) continuum and line emission by ionised gas, the intensity of which can be expected to vary much more slowly and with smaller amplitude than the UV continuum; (3) the direct output of stars; and (4) the IR excess. Coordinated optical to IR photometry can be combined with spectral photometry to determine the spectra of the UV and IR source components.

Spectra of IR sources

Separation of the UV and IR components of the nuclear source in III Zw 2 should be relatively straightforward, as the nonthermal (optical-UV) continuum is presumably of much higher luminosity than any stellar contribution (the source is sometimes classified as a QSO because of its compact appearance on plates). We assume that the spectra of the continuum components do not change during variations and that the continuum flux at B is entirely from the nonthermal source. The first of these assumptions is suggested by our photometry but needs to be tested by additional observation; a justification for the second follows from our calculations below. With these assumptions, it is possible to estimate the relative contributions of the nonthermal and IR sources at *J*, using the fact that the variation at *J* is intermediate between that at *B* and *K*, and thus that *J* includes contributions from both the nonthermal and the IR sources. A crude estimate would assume that the nonthermal source contributes negligibly to *K*. This estimate can be refined in an iterative way by extrapolating the nonthermal continuum as a power law from *B* and *J* to *K*, determining the variation of the IR source alone at *K*, and repeating the separation at *J*. For 1 May 1979 one finds that the nonthermal fluxes at *J*, *H*, and *K* are respectively 7.3, 8.0 and 9.0 mJy, in reasonably good agreement with a simple extrapolation of the optical-UV continuum¹⁰. The power law fit to the *B* and *J* fluxes has a slope of -0.4 . The IR

source produces fluxes at *J*, *H* and *K* of 2.8, 5.3 and 14.2 mJy, respectively, and a power law fit from *J* to *K* would have an index of ~ -3 .

The very rapid fall in the spectrum of the IR source towards shorter wavelengths was already indicated for several other Seyfert galaxies by spectral inflections near $1 \mu\text{m}$ (ref. 11) and, for NGC1275, 4151, 5548 and 7469, by an approximate separation of the spectrum into stellar, variable UV, and IR components.¹¹ The separation of source components by accurate, coordinated measurements of variability from the UV into the IR should be more reliable than these other two methods. However, for 3C120, NGC1275 and NGC5548 our data are not yet sufficiently extensive for such an analysis. The variability of NGC4151 will be discussed in detail elsewhere.

Role of dust in Seyfert galaxy nuclei

The slope of the spectrum of the IR component in III Zw 2 corresponds to a colour temperature between 1.25 and $2.2 \mu\text{m}$ of 1,500 K, corresponding closely to the maximum temperature at which interstellar grains can survive. If the near IR flux is

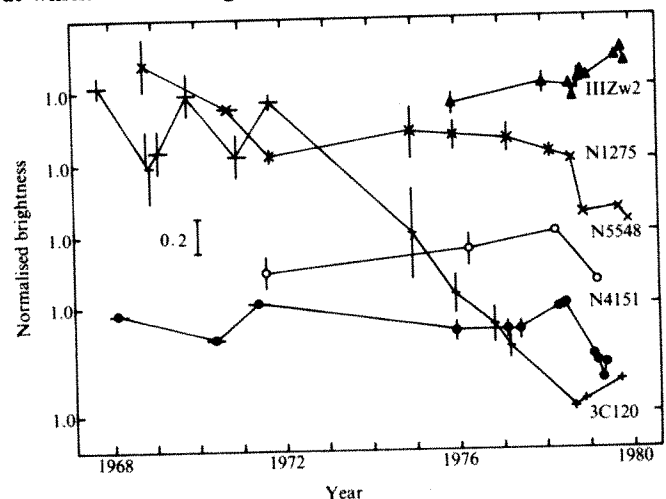


Fig. 1 IR Variability of five Seyfert galaxies. Data reported here have been combined with those summarised in ref. 5. The spectrally averaged IR brightness for each galaxy has been normalised to 1.0 near the middle of 1978. The bar at the beginning of 1970 shows the amplitude of a variation of 20% of the normalisation value. When measurement errors exceed the sizes of the symbols, they are indicated with vertical bars. When measurements over an extended period have been combined into a single point, the total period of measurement is shown with a horizontal bar.

radiated by hot, interstellar grains certain limitations can be placed on the size of the emitting region. Assuming the distance is as indicated by a cosmological interpretation of the redshift of $z = 0.089$ with $H_0 = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}$, the radius of a 1,500 K blackbody that can generate the observed near IR fluxes is $\sim 0.2 \text{ pc}$. If the central UV source has a luminosity of $\sim 10^{12} L_\odot$ (see below) and the absorptivity of the grains is neutral between the UV and IR, the grains will reach an equilibrium temperature of 1,500 K at a distance of $\sim 0.2 \text{ pc}$ from the central source. The IR source must be somewhat larger than either of these estimates, as the bright optical and UV flux from III Zw 2 demonstrates that the source is optically thin (at least towards the Earth) and most plausible dust constituents have greater UV than IR absorptivity, raising the equilibrium temperature for a given distance from the central source. However, the dependences on radius are sufficiently steep that the size should be close to the lower limits estimated above: a size of $\sim 0.6 \text{ pc}$ would require an optical depth of only 10% and UV absorptivity 16 times that in the IR. A thermally re-radiating source 0.6 pc in radius would smooth out all variations of the UV flux and delay them with a time constant of $\sim 2 \text{ yr}$. This estimate agrees well with the brightening of III Zw 2 in the IR in September 1979, $\sim 2 \text{ yr}$ after the onset of a powerful optical-UV and radio outburst¹². Further observations in the IR can test this association.

There is already evidence that most Type 2 and those Type 1 Seyfert galaxies with steeply rising IR spectra, such as NGC4151 and NGC5548, emit thermally in the IR¹¹. Among Type 1 Seyfert galaxies, III Zw 2 has one of the most slowly rising spectra into the IR. From its behaviour, re-radiation in the IR by dust is an important component of its spectrum also.

The luminosity of the IR source in III Zw 2 is $\sim 4 \times 10^{11} L_\odot$ (ref. 11). From the strength of $H\alpha^{13}$, the luminosity in the Lyman continuum must be $\sim 2 \times 10^{11} L_\odot$. If the nonthermal continuum can be represented by a power law of index -0.4 from $1 \mu\text{m}$ to the Lyman limit, the luminosity over this spectral range is $\sim 6 \times 10^{11} L_\odot$. Therefore, a significant proportion of the UV continuum of the nonthermal source is absorbed by dust and re-radiated in the IR. The ratio of $H\alpha$ to IR luminosity for III Zw 2 is among the largest for Seyfert galaxies; for most of these

Table 2 Additional IR photometry

Source	Date (UT)	J	H	K	L	(IR)	$\delta(\text{IR})$
III Zw 2	9.10.78	8.6	11.1†	21.4		0.94	0.02
	11.2.78			23.2*		1.04	0.06
	11.19.78	10.8	13.8	23.9		1.08	0.02
	11.12.79	8.9	14.8	28.3	46‡	1.20	0.02
NGC4151	4.24.78	119†	173	231	361	0.97	0.02
	5.22.78		165†	229	392	1.00	0.02
	5.30.78	118†	168	227		0.96	0.02
	6.18.78	126	173†	238		1.00	0.02
	2.9.79	92	124†	166		0.72	0.01
	4.13.79	81‡	104†	136		0.60	0.01
	4.19.79			128		0.56	0.02

Fluxes, aperture, and errors as in Table 1. $0 < \text{errors} < 2\%$ unless otherwise indicated.

* $4\% < \text{errors} \leq 6\%$. † $2\% < \text{errors} \leq 4\%$. ‡ $6\% < \text{errors} \leq 10\%$.

sources the role of dust will be relatively larger than in III Zw 2. From the time scale of the IR variations for all five galaxies we have studied, at least part of this dust lies within 0.1–0.6 pc from the nonthermal sources, depending on the galaxy. The dust therefore lies within or at the outer boundary of the region responsible for the broad emission lines in the Type 1 galaxies.

Conclusions

From accurate monitoring of the near IR fluxes of five Seyfert galaxies, we conclude:

Most or all Seyfert galaxies that vary in the optical-UV also vary in the near IR.

The IR variations are not simultaneous with the optical-UV ones, but have the characteristics of dust reradiating the absorbed nuclear UV continuum.

From the time scale of the IR variability, the dust lies near the gas producing the broad emission lines in Type 1 Seyferts.

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The representation of colours in the cerebral cortex

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New insights into how colour is represented in the cerebral cortex and what variables govern the responses of single cortical colour-coded cells have been gained by the discovery of specific visual cortical areas rich in colour-coded cells.

OUR ability to see an almost infinite variety of colours depends on the presence, in the retina, of only a limited number of receptor types. That the number must be limited was first proposed by Thomas Young. He wrote¹, "Now as it is almost impossible to conceive each sensitive point on the retina to contain an infinite number of particles, each capable of vibrating in perfect unison with every possible undulation, it becomes necessary to suppose the number limited, for instance to the

three principal colours, red, yellow and blue". The identification of three cone pigments in the primate retina^{2–5}, each with a broad response curve, but each having a maximal sensitivity to a different part of the visible spectrum, was a striking confirmation of Young's theory. In its simplest form, this theory supposed that the colour of each 'point' in the field of view is determined by the relative responses of the three cone pigments at a corresponding 'point' in the retina and that this relative response is determined

by the amount of light in each waveband coming from that 'point'. Most colour scientists since Young have, however, assigned a role to more central nervous interactions in accounting for colour perception, and especially for colour constancy^{7,8}. This refers to the persistence of the colour of objects or of surfaces when viewed in lights of different spectral composition, such as daylight and light produced by a tungsten filament bulb. It cannot be explained in terms of energy-wavelength relationships. Other explanations have therefore been sought and, in most, the cerebral cortex has been given a dominant, but neurophysiologically a vague and ill-defined, role. Helmholtz thought learning and judgement to be critical^{6,9}. Hering considered memory to be important¹⁰. Other factors, such as adaptation, have also been suggested⁸. Land^{11,12} in his retinex theory, has sought to explain colour vision (including colour constancy) in computational terms which are essentially independent of energy, surroundings, adaptation, contrast and all psychological factors while being critically dependent on the cortex.

What role, then, does the cortex have in colour perception and how are colours represented there? We decided to explore the responses of single colour-coded cells in the cortex of the rhesus monkey, an animal close to man, with the hope of gaining some insight into these questions.

Wavelength sensitivities of colour-coded cells in the cortex

Anatomical^{13,14} and functional¹⁵⁻¹⁷ studies of rhesus monkey prestriate visual cortex have shown that the different areas within it have remarkably different populations of functional cells, thus leading to the theory of functional specialisation in the visual cortex^{17,18}. One such specialisation occurs in the fourth visual areas which, unlike other prestriate areas, are rich in colour-coded cells¹⁵⁻¹⁹. Since the fourth visual areas are the highest cortical areas to which the analysis of colour has been traced, both anatomically¹⁴ and physiologically^{16,19}, we began by studying the responses and wavelength sensitivities of cells there. Chief among the properties of these areas is the relatively large size of the cells' receptive field^{16,19} (when compared to field sizes at equivalent eccentricities in the striate cortex) and the concentration of field positions in the central 20-30° of the retina. One of the most striking features is the absence of a discernible retinotopic organisation such as that found in the striate cortex²⁰, since field positions in long oblique penetrations move in an unpredictable manner which bears no obvious relation to retinal topography.

Using conventional anatomical and electrophysiological techniques¹⁸, single cells in the fourth visual areas were isolated in the anaesthetised monkey and their receptive fields plotted. Their wavelength response curves (action spectra) were determined by establishing the threshold response at different wavelengths¹⁶. Figure 1 shows some representative narrow-band action spectra. It is evident that cells in V4 can be selective to narrow parts of the visible spectrum. Just how narrow these are is shown in Fig. 2. This is a plot of peak sensitivity against wavelength. Throughout most of the spectrum, bandwidths range from 10 to 50 nm, with many having a bandwidth at half maximum of between 10 and 20 nm. Thus the response of cells in V4 can be said to approximate more closely what may be termed responses to individual colours, or hues, than anything seen in more antecedent parts of the visual system^{21,22}.

One can now go further and ask whether cortical colour representation limits the peak sensitivities of cells to certain parts of the visible spectrum, as in the retina. To rephrase Young¹, "is it necessary to limit the peak sensitivities, for instance to the three principal colours, red, yellow and blue". Figure 3 shows the answer to be no. Between them, the peak sensitivities of these narrow band cells cover almost the entire visible spectrum. Superimposed on this wide distribution, however, is a clustering, most cells having their peak sensitivities at 480 nm (blue), 500 nm (green) and 620 nm (orange-red). Hence these are the regions of the spectrum most strongly represented in the cortex. Purple, an extraspectral colour, is also

strongly represented. Finally, it is noteworthy that, as in the striate cortex²³, no cells have peak sensitivities in the psychophysically least saturated part of the visible spectrum, that is in the 560-570 nm region²⁴, roughly where the long-wave pigment has its maximal absorption. Why this should be so is not clear. With further experiments, cells with peak sensitivities in this region may be found. So far, however, it seems that the cortical representation of this part of the spectrum is poor.

Mapping of colours in the cortex

Given such narrow band cells and given the clustering of peak sensitivities in the blue, green, purple and red, it is interesting to ask how these colours are mapped in the cortex. From micro-mapping experiments, there seems little doubt that cells with particular colour preferences are grouped together in the cortex of V4^{16,19}. This is especially well seen in perpendicular electrode

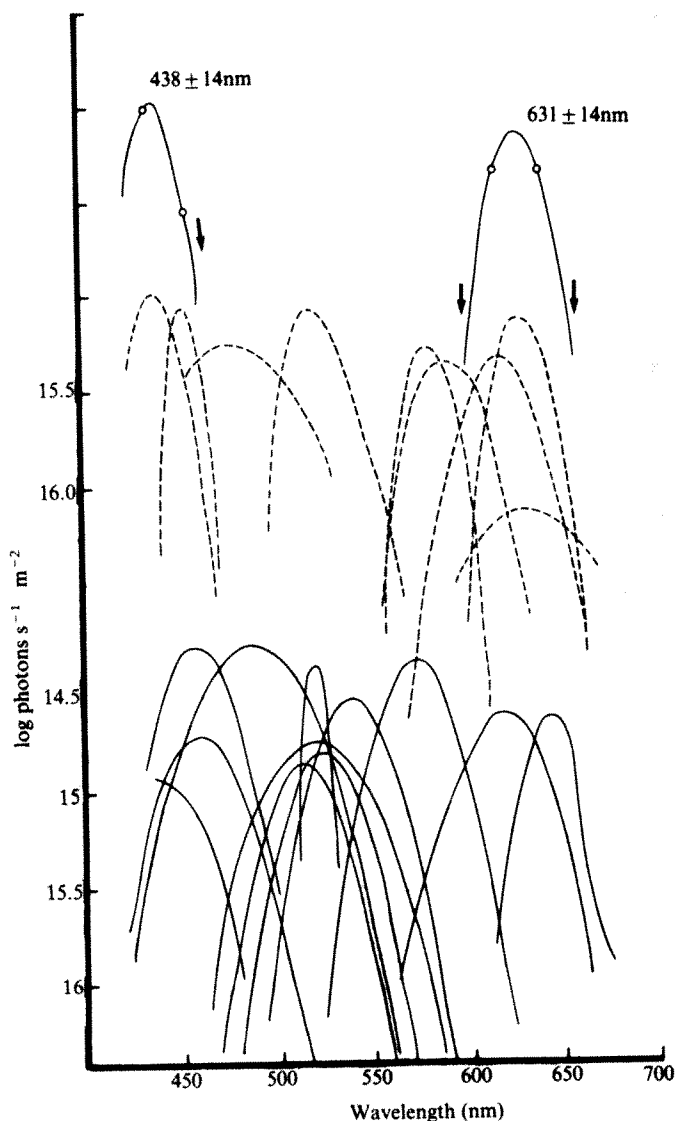


Fig. 1 Representative action spectra (wavelength selectivities) for some narrow-band cells of the fourth visual areas (V4) of monkey cortex. Action spectra were obtained by using neutral density filters and determining the minimum intensity at every wavelength to which there was a response. The upper two spectra show the responses obtained and the manner of drawing the curves to determine peak sensitivity and bandwidth. Arrows indicate there was no response at the highest intensities available. In the conventional way, curves were drawn through the experimental points by using a family of template curves, all of which were parabolas, and judging by eye which one gave the best fit. Action spectra in discontinuous lines are those of cells inhibited by light of the relevant wavelengths, those in continuous lines represent action spectra of cells excited by the relevant wavelengths.

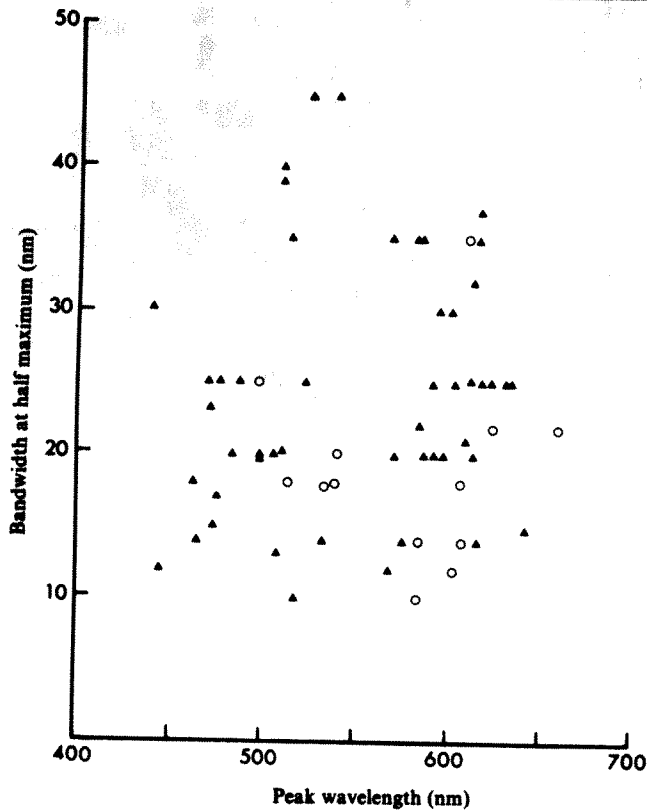


Fig. 2 A plot of peak sensitivity against bandwidth at half the maximum sensitivity for 63 action spectra obtained from 50 cells. \blacktriangle , Excitation spectra; \circ , inhibitory spectra.

penetrations^{16,19}. In oblique penetrations, the changes in colour preferences of successive cells, with electrode distance, are obvious and, in some, such changes are orderly. A fortunate oblique penetration, one of several, is illustrated in Fig. 4. The electrode travelled a total distance of 1,300 μm . Receptive fields of cells were large, and the shift in receptive field position was apparently random. However, the arrangement of cells with respect to one another was far from random functionally. The first three cells responded with an increase of their maintained discharge to blue, and with a decrease to red light. The next five cells were excited by green and inhibited by purple. These were followed by a cell responding to white light only. Finally, the last two cells were excited by red light and inhibited by blue. Action spectra were plotted for most of the cells. It was found that, throughout this progression, peak excitatory sensitivities were displaced from the short to the long end of the spectrum (except for the jump back from the long to the short part from cell 6 to 7). The peak inhibitory sensitivities, by contrast, were displaced in precisely the opposite direction, going through purple, the complementary, to green. Although the peak opponent sensitivities do not all cross at the white point W on the x-y chromaticity chart (Fig. 4) the order apparent when peak sensitivities are plotted on that chart is impressive and suggests that colours may be mapped in an orderly way in the cerebral cortex. The strong representation given to blue, green, purple and red is also evident in this penetration.

A study of the responses of colour-coded cells in the cortex using Land's retinex experiments

The results given above, as well as previous findings^{16,19}, attest to a high degree of order in the representation of colour in the cerebral cortex: first in generating cells with narrow wavelength selectivities and segregating them into specific cortical areas^{17,18}, then in mapping the retinal surface in these areas in a manner radically different to the retinal map in other prestriate areas¹⁴,

finally in mapping colours in an ordered way. The very segregation of cells sensitive to colour into separate cortical areas suggests that the principles governing the representation of colour in the cortex must be very different from those governing the representation of, say, form or depth. What are these principles?

Edwin Land, the originator of retinex theory, suggested to me that the responses of the cells described above were sufficiently interesting to warrant a new kind of study the purpose of which would be to ascertain the extent to which such responses correspond to, and obey, the rules of colour perception. The simple 'Mondrian' experiments in colour vision²⁵ were designed by Land to answer the classical question of why colours change so little when the wavelength-energy composition of illumination on objects is changed markedly. By using rectangles of arbitrary shape, size, surround and colour, and by choosing matte papers which reflect a constant amount of light in all directions, he created a multicoloured complex image with no recognisable objects. By illuminating this abstract scene with three bands of wavelengths whose relative energies were variable, he brought into focus the fact that colour sensations are essentially independent of energy and also independent of memory, learning, judgement, surroundings and adaptation. These perceptual experiments were so readily adaptable to electrophysiological ones that it seemed interesting to learn whether there were any cortical cells whose responses would correspond to the sensation of colour produced by the rectangles of the Mondrian display. I am much indebted to Land in the execution of the experiments described below.

Provided with this article are three filters, with cutoff points as follows: short wave, 386 and 493 nm (peak transmittance 432 nm); middle wave, 492 and 580 nm (peak transmittance 528 nm); long wave, 592 nm (peak transmittance greater than 660 nm). Each filter was chosen with a bandwidth such that an observer cannot see variegated colours through it. If one were to view a Mondrian display or a similar multicoloured scene through the red filter one would see a pattern which would be the same in disposition for all three sets of cones, even though the energy available to be absorbed by the middle-wave set and short-wave set of cones will be relatively greatly diminished. Thus all the patches which were variously coloured in unfiltered illumination will be seen as a series of light and dark areas against a reddish wash and the area which was red in unfiltered illumination will be seen as one of the lightest. Other areas such as yellow and white will also appear very light and an observer would not find it possible to predict which, among the areas that appear very light, will be red when the display is later viewed in unfiltered light. For the red area, the transition from this high lightness to a deep red in unfiltered illumination can be better understood by examining the Mondrian again, first through the green filter and after that through the blue filter. For either of these filters, as opposed to the red one, the area which is deep red in unfiltered illumination will be very dark. Thus it is as if the

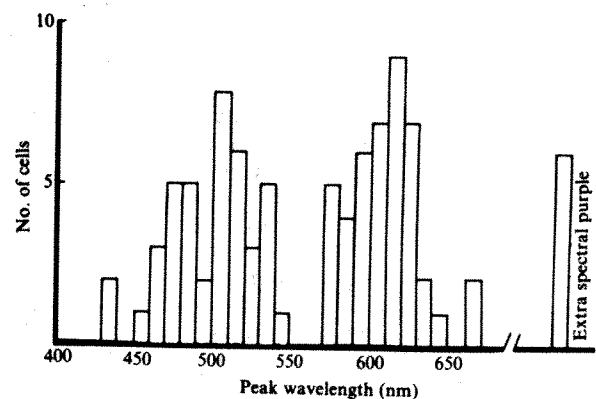
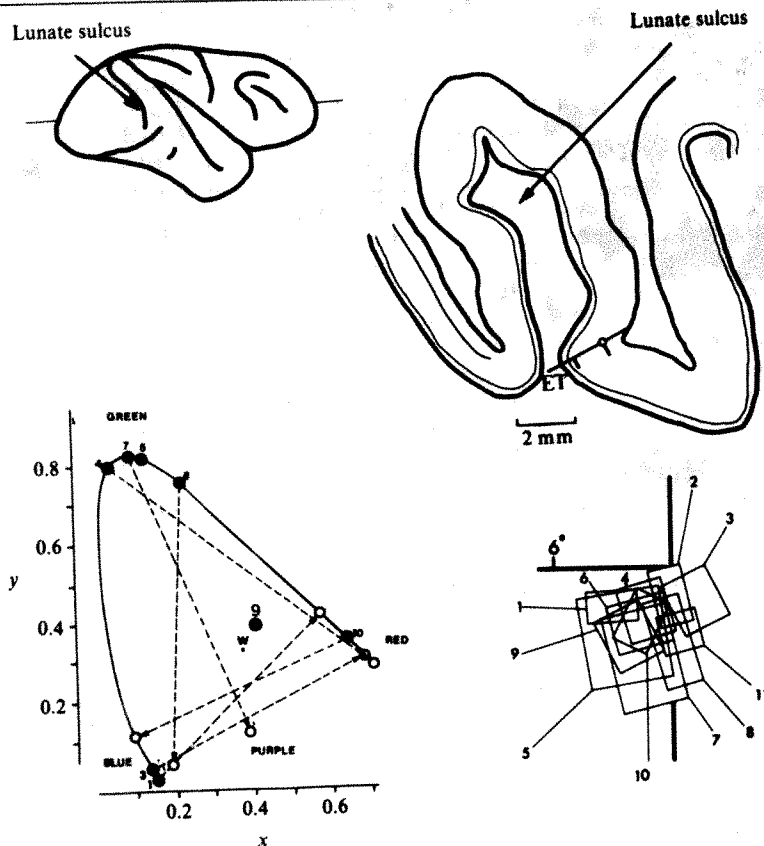


Fig. 3 Histogram of the distribution of peak sensitivities of narrow-band cells in the fourth visual areas (90 spectra obtained from 62 cells).

Fig. 4 Reconstruction of an oblique electrode penetration (ET) through the cortex of V4, made at the level indicated on the surface drawing of the brain. Receptive fields of successive cells are shown to the lower right. As in longer penetrations, shifts in receptive field position bear no obvious relation to retinal topography. The peak sensitivities of the narrow-band cells, determined from their action spectra, are entered on the x - y chromaticity chart to the left. The position of cell 9 (white) on the chart was determined from measurements of the spectral distribution of the light source and the spectral reflection coefficients of the screen. ●, Peak excitatory sensitivity; ○, peak inhibitory sensitivity. Cell 5 was lost before the inhibitory part of the action spectrum could be determined.



deep red of the area in unfiltered illumination is the consequence of the simultaneous presence of the high lightness as seen through the red filter and the two low lightnesses as seen through the green and blue filters. In general three completely independent patterns in terms of lightness and darkness will be seen through the three filters respectively. Each of these patterns as seen through one of the coloured filters will not change if a neutral filter of low, medium or rather high density is superimposed on the colour filter. It is this basic phenomenon which manifests itself as colour constancy when the relative flux in the three illuminators is altered (see below). Are there any colour-coded cells in the cortex whose response is similar to the visual experience produced by viewing Mondrian displays in variable illumination?

If to determine a red region within the field of view as being red it is necessary to illuminate the entire scene, not only by long-wave, but by middle- and short-wave light as well (and hence stimulate all three sets of cones over extended retinal regions), it follows that somewhere along the visual pathways colour-coded cells must give their optimal response when a display containing a red region, say, is illuminated not only by long- but by middle- and short-wave light as well. This was studied in the following way. Once the receptive field and the action spectrum of a cell was plotted, a multicoloured Mondrian display was placed on the screen in such a way that for a cell, say, that responded to blue only, a blue part of the Mondrian covered its receptive field. The Mondrian display was made of special matte papers, selected to have a minimum reflectance higher than 10% for any part of the visible spectrum. The display could be illuminated by three projectors, each equipped with a 750-W tungsten filament bulb and with sharp cut band-pass filters, one passing long, one middle and one short waves. The flux from each projector could be set by a rheostat. The illuminating filters were selected by Land to minimise the diversity of colour sensations from the array of coloured papers when only one projector was turned on and, while satisfying the first condition, to transmit as wide a band of wavelengths and as much light as possible²⁶.

Figure 5 shows the responses of a cell with a narrow action spectrum, responsive to red exclusively. It had no evident opponent input, either in the centre or in the surround. With the

Mondrian display in full illumination, it responded only when the red area was put in its receptive field. However, illuminating the display with long-wave light alone (equivalent to viewing it through the red filter) was totally ineffective in activating the cell. It was also unresponsive to illumination with middle- or short-wave light alone. But when all three lights were switched on simultaneously the cell gave a brisk response. This was the very condition in which the area in the cell's receptive field appeared a vivid red to human observers. Hence one's curious impression that the firing of the cell was in fact the sensation of red. In further experiments, identical responses were obtained for cells responding exclusively to green and to blue.

Table 1 Mondrian areas

Cell	White	Grey	Red	Green	Blue	Magenta	Yellow
'Red' cell	—	—	+	—	—	—	—
'White' cell	+	—	—	—	—	—	—
'Green' cell	—	ND	—	I. + II. +†	—	—	ND
'Red' cell	—	—	+*	—	I. — II. —‡	—	ND

The response of four cells to different areas on the Mondrian display when the energies coming from each area were identical. +, Presence of a response; —, no response. Unless otherwise stated each area of the display was arranged to send 69 milliwatts per steradian per square metre of long-wave light, 31 $\text{mW sr}^{-1} \text{m}^{-2}$ of middle-wave light and 5 $\text{mW sr}^{-1} \text{m}^{-2}$ of short-wave light. ND indicates that the area was not studied for that cell.

* There was only 21 $\text{mW sr}^{-1} \text{m}^{-2}$ of middle-wave and 4 $\text{mW sr}^{-1} \text{m}^{-2}$ of short-wave light at this reading.

† On a second reading for this cell, energies were arranged so that the area sent 120 $\text{mW sr}^{-1} \text{m}^{-2}$ of long-wave, 40 $\text{mW sr}^{-1} \text{m}^{-2}$ of middle-wave and 5 $\text{mW sr}^{-1} \text{m}^{-2}$ of short-wave light. There was a response.

‡ On a second reading, energies were arranged so that there was 15 $\text{mW sr}^{-1} \text{m}^{-2}$ of long-wave, 17 $\text{mW sr}^{-1} \text{m}^{-2}$ of middle-wave and 8 $\text{mW sr}^{-1} \text{m}^{-2}$ of short-wave light coming from that area. The cell did not respond.

While the responses of the cell illustrated in Fig. 5 are impressively clear, the same basic phenomenon was observed in many other cells, even if the responses were often not as sharp. With all such cells, however, the effects of switching off two of the three projectors, leaving only the one of the cell's preferred colour, was always the same; it led to a dramatic fall in the cell's firing rate. Figure 6 A-C shows recordings from groups of cells, all of them responsive to red alone. With the red area of the Mondrian display placed in the receptive field, switching on long wave light alone was ineffective in activating the cells of (A), gave a weak response from those of (B), and a powerful response from those of (C). In the latter, the discharge rate of the cells fell sharply after the initial outburst. Adding the middle- and short-wave lights (which, by themselves, were ineffective in activating the cells) produced the same result for all three groups—a pronounced increase in firing rate. Switching them off also produced the same result—an almost complete cessation of firing.

It is remarkable that for a cell, such as that of Fig. 5, middle- and short-wave lights should, by themselves or in combination, be totally ineffective in activating the cell and yet be instrumental in driving it in the presence of long-wave light—precisely the condition needed for a human observer to experience the sensation of a vivid red in such a multicoloured display. If these cells were themselves comparing the 'lightness records' of that area at the three wavebands, then one might have expected their

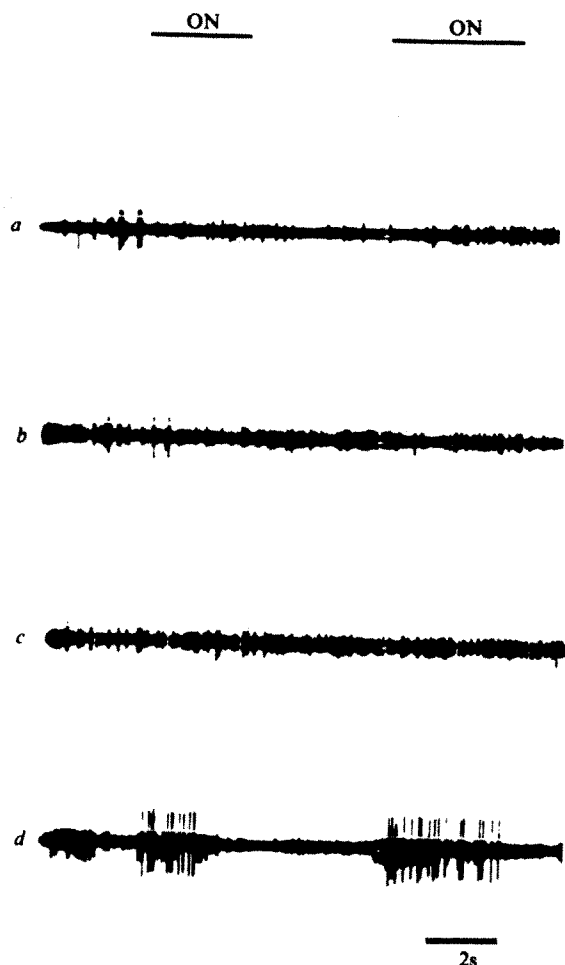


Fig. 5 The responses of a narrow-band red-orange sensitive cell (peak sensitivity 620 nm) to the red area of the Mondrian display when the display was illuminated by *a*, long-wave; *b*, middle-wave and *c*, short-wave light. In *d* the response to illuminating the display with all three projectors simultaneously is shown. To provide long-wave light, a Wratten 29 filter was placed in the light patch. The middle- and short-wave projectors had sharp cut band-pass filters with dominant wavelengths at 525 nm and 440 nm respectively.

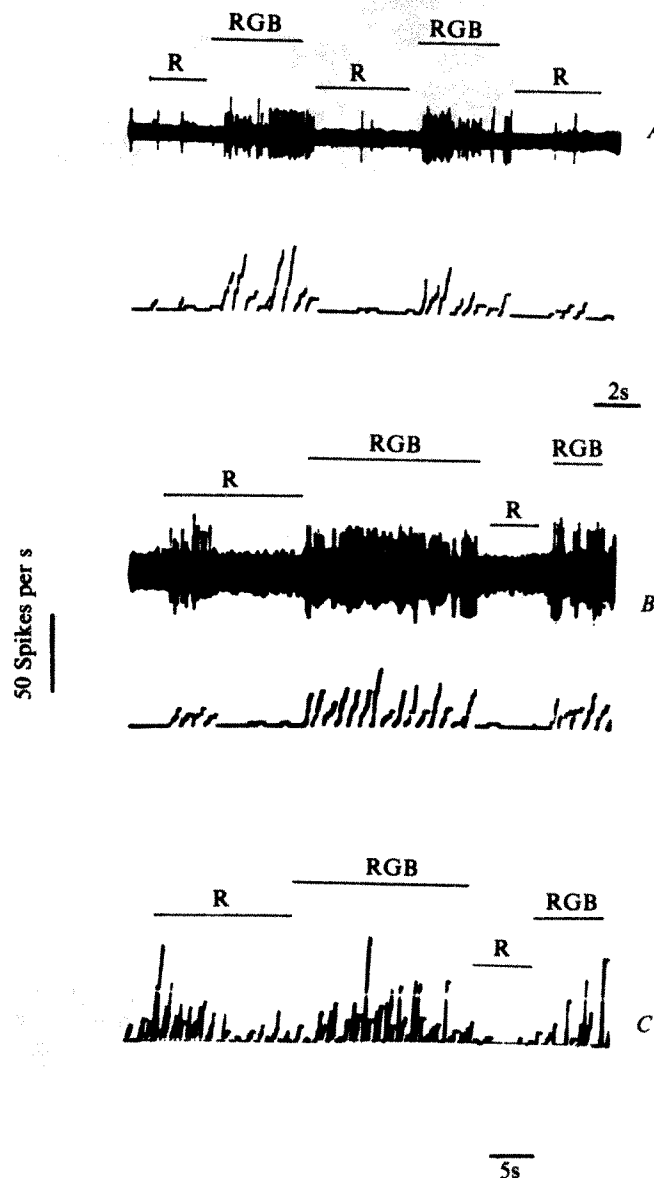


Fig. 6 The responses of three groups (A, B and C) of red-sensitive cells to the red area on the Mondrian display, when the display was illuminated with long-wave light alone (R) and when illuminated with long-, middle- and short-wave light (RGB). In the first two groups, the upper trace is the spike discharge of the cell and the lower trace the frequency of that discharge. For group C only the frequency of discharge is shown.

activity to be influenced in some way by light at each waveband. That the burst of activity occurs only when all three projectors are switched on simultaneously suggests that, if such a comparison is indeed the basis of the response, it must occur at antecedent levels. We have no clear notion whether 'lightnesses' at each waveband are registered by individual cells or whether more subtle interactions, requiring the activity of pools of neurones, is needed. It would therefore be premature to hazard a guess as to what level this might occur at. But V4, with the repetitive representation of each region of the retina in it^{14,16}, is as good a candidate as any. In this context, I emphasise that not all colour-coded cells in V4 behave in this 'experiential' way. Some, with action spectra restricted to the long end of the spectrum, behave much more simply, responding whenever long-wave light is switched on, no matter what part of the Mondrian display is in the receptive field. The role of these cells, and the wiring from them to the ones described above, remain to be established.

If a cell, such as that of Fig. 5, responsive exclusively to the red part of the Mondrian display when the display is trichromatically

illuminated, will nevertheless not respond to it, or do so weakly, when the same display is illuminated by long-wave (red) light alone, it becomes obvious that these responses cannot be dictated by energy-wavelength relationships. But it seemed worth repeating with these cells the experiment that Land reported on human subjects¹². Specifically, how would the cell of Fig. 5, say, respond if the amount of long-, middle- and short-wave light coming from that area on the Mondrian were made identical to the amounts of those lights coming from another part of the Mondrian, say the white, to which the cell was unresponsive. To do this, the amount of long-, middle- and short-wave lights coming off the white area were read off, one by one, using a Gamma Scientific telephotometer fitted with an equal sensitivity filter²⁶. Then, going to the other areas on the Mondrian, the amounts of all three lights were adjusted so that, from each, the identical triplet of energies reached the eye. Table 1 below shows how four narrow band cells on the Mondrian were made to send identical triplets of energy and put in the cells' receptive fields. It is clear that the responses of these cells could be correlated with colour alone and were independent of flux. For the green cell of Table 1, the green area of the Mondrian display was placed in the receptive field and energies adjusted so that three times more long- than middle-wave light reached the eye from it. The area still appeared green to me, a normal trichromat, and the cell gave a good response. Again, for the red cell of Table 1, the blue area was placed in its receptive field and energies adjusted so that while the area still appeared blue, it nevertheless sent twice as much long and middle, than short wave, light to the eye, and yet the cell still did not respond to it. The independence of these responses from flux is striking.

Figure 7 shows an even more remarkable response, that of a cell responding exclusively to white (of which I have encountered a few examples). When energies were made equal so that each area, when placed in the cell's receptive field, was sending an identical triplet of energies, the cell still responded only to the white area.

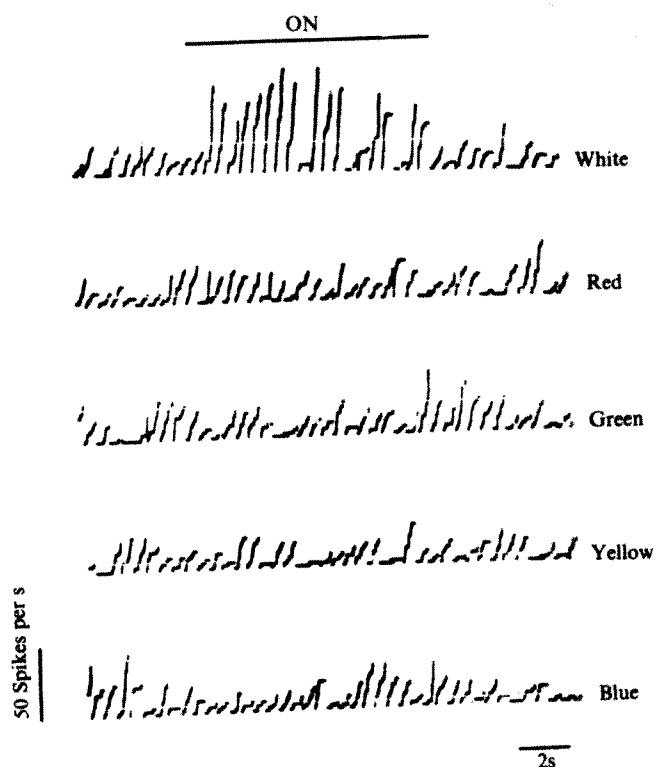


Fig. 7 The response of a cell selective to white when different areas on the Mondrian display were made to send an identical triplet of energies as that coming from the white area and placed in the cell's receptive field.

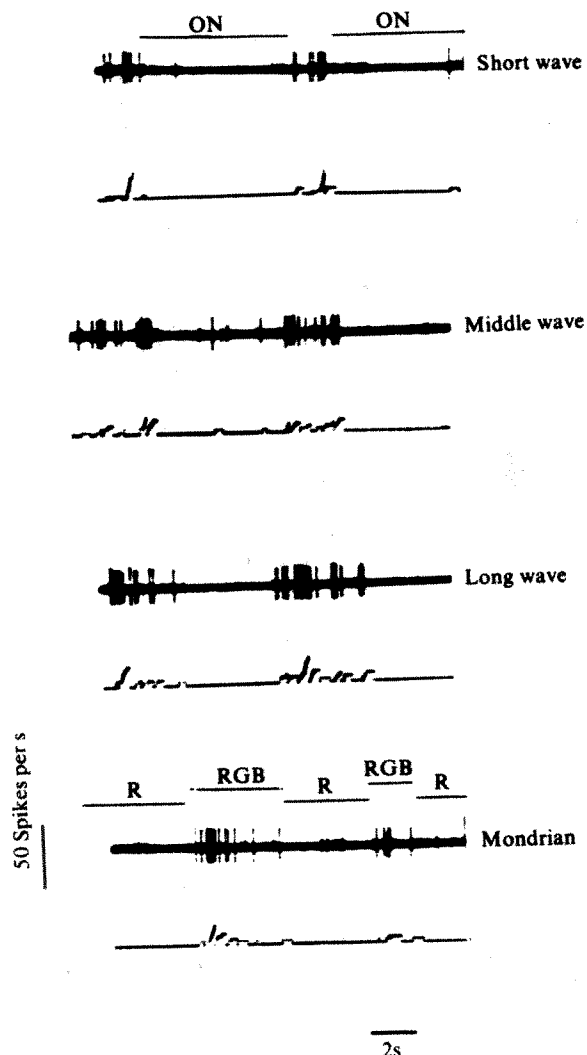


Fig. 8 Upper three traces show the response of a cell to illuminating its receptive field diffusely with short-, middle- and long-wave lights separately. The lower trace shows what happens when the red area of the Mondrian display is placed in the cell's receptive field and the entire display illuminated, first with long-wave light alone (R) and then trichromatically (RGB). The energy from the red area when the display was illuminated with long-wave light alone was $540 \text{ mW sr}^{-1} \text{ m}^{-2}$; when trichromatically illuminated it was $560 \text{ mW sr}^{-1} \text{ m}^{-2}$.

Observations such as these imply that for the single cell, just as for perception, the composition of light in terms of energy-wavelength relationships may be of little importance. Cells specifically responsive to flux, either by increasing or decreasing their firing rate, exist in the prestriate cortex (my unpublished results). However, explaining such responses in terms of energy may be wrong, as the behaviour of the cell of Fig. 8 shows. This V4 cell had a high maintained discharge which was abolished by shining short-, middle- or long-wave light diffusely onto the screen facing the animal. Switching the lights, especially the long wave one, off, led to a vigorous discharge. Superficially, then, the cell behaved as if it decreased its firing rate in response to an increase in flux. But its response using the Mondrian display contradicted this first impression. With the red area of the Mondrian display placed in its receptive field, the entire display was flooded with long-wave light (equivalent to viewing the red area of the display through the red filter). Perceptually, the red area now appeared very light, and the cell's maintained discharge was abolished, as expected. Now, adding short- and middle-wave light to the long-wave (equivalent to viewing the red area of the display¹⁵ in full illumination) and thereby increasing the energy further still led, dramatically, to an

increase in firing rate (Fig. 8). Perceptually, the effect of illuminating the Mondrian display with all three lights was to make the red area appear not only a vivid red, but also darker than it had appeared when the display was illuminated by long-wave light alone. In brief, the responses of the cell could be correlated well with human perception and were dramatically illustrated to be independent of flux.

A new neurophysiology of colour vision

The discovery of specific visual areas rich in colour-coded cells has brought us a step closer to understanding the physiology of colour vision and the nature of colour representation in the cortex. It may also provide a new approach to the study of colour vision. First, functional mapping experiments (see Fig. 4) do not reveal a topographically organised 'map' in relation to the retina. Instead they often show a representation of distinct functions (in this instance, colour) with an arrangement of the cells appropriate to that function and not necessarily following any simple topographic relation to the 'visual field'. No doubt the representation of functions also determines the pattern of anatomical connections of these areas^{13,14}. It may therefore be more meaningful to ask for these and other visual areas, how a

function is mapped, rather than how the 'visual field' is mapped.

Second, the responses of the cells reported here correspond so well with the sensation of colour that we can now, perhaps for the first time, apply identical techniques for the study of perceptual responses and those of individual cortical cells. Whereas it may be difficult to equate directly the responses of orientation selective cells with the perception of form, or of depth detecting cells with the perception of distance, there is little difficulty in equating the perception of colour with the response of the individual colour-coded cells described here. It seems timely, therefore, to apply the rules of colour perception in natural situations to the study of single cells, although we are, of course, a long way from knowing whether the cells described here are 'experiential' ones, enabling us to see the many and varied colours around us.

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Transforming activity of DNA of chemically transformed and normal cells

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DNA fragments of chemically transformed and normal avian and murine cells induce transformation of NIH 3T3 mouse cells with low efficiencies. High molecular weight DNAs of cells transformed by DNA fragments induce transformation with high efficiencies in secondary transfection assays. It thus seems that endogenous transforming genes of uninfected cells can be activated and efficiently transmitted by transfection. These results are consistent with the hypothesis that normal cells contain genes that are capable of inducing transformation if expressed at abnormal levels.

SUBSTANTIAL evidence indicates that highly oncogenic retroviruses are recombinants between non-transforming viruses and normal cell genes. Examples of transforming viruses that have apparently originated by recombination with different cell genes include avian sarcoma viruses¹⁻³, avian myelocytomatosis virus^{4,5}, avian myeloblastosis virus⁵, avian erythroblastosis virus⁵, Moloney sarcoma virus^{6,7}, Abelson leukaemia virus⁸, Kirsten and Harvey sarcoma viruses⁹ and feline sarcoma viruses¹⁰. In the case of avian sarcoma viruses, it has been demonstrated that genetic information related to the viral transforming gene (*src*) is encoded in the genomes of many vertebrate species³. In addition, uninfected avian and mammalian cells contain a normal cell protein (p60^{src}) that is closely related to the protein encoded by the avian sarcoma virus *src* gene (p60^{src})¹¹⁻¹³. The amount of p60^{src} present in avian

sarcoma virus-transformed cells seems to be at least 100-fold higher than the amount of p60^{src} present in uninfected cells¹¹⁻¹³. These observations suggest that viral transformation may result from overproduction of normal cell proteins as a consequence of the insertion of normal cell genes into a viral genome in a manner permitting their efficient expression. If this is the case, it also seems plausible that the transforming genes of retroviruses represent only a subset of the normal cell genes that are potentially capable of inducing transformation if expressed at higher than normal levels. The present experiments indicate that DNAs of both chemically transformed and normal uninfected cells are capable of inducing transformation on transfection. The results thus provide direct support for the hypothesis that potential transforming genes are encoded in the genomes of normal avian and mammalian cells.

Transformation by sonicated DNA fragments of uninfected cells

The transforming activity of uninfected cell DNAs was assayed by transfection of NIH 3T3 mouse cells. These cells are transformable by direct integration of DNAs of avian sarcoma viruses¹⁴, avian acute leukaemia viruses¹⁵ and murine sarcoma viruses^{7,16}. In addition, NIH 3T3 cells are transformable by subgenomic fragments of murine⁷ and avian¹⁵ sarcoma virus DNAs. The efficiency of transformation by subgenomic fragments of avian sarcoma virus DNA, which contain *src* but apparently lack the normal viral transcriptional promoter, is 100–1,000-fold lower than the efficiency of transformation by intact virus DNA¹⁵. This reduced efficiency is thought to represent the probability of integration of these fragments at a site adjacent to a transcriptionally active promoter in the DNA of the recipient cells. A similar efficiency of transformation by fragments of normal cell DNAs might therefore be expected if transformation could occur by integration of normal cell genes at a site in recipient cell DNA leading to their increased expression.

The donor DNAs used in these experiments were extracted from methylcholanthrene-transformed quail cells (QT-6 cells)¹⁷, methylcholanthrene-transformed BALB 3T3 mouse cells (MC5-5 cells)¹⁸, normal chicken embryo fibroblasts and non-transformed BALB 3T3 and NIH 3T3 mouse cells. Both high molecular weight (MW) and sonicated DNAs were used in transfection assays to investigate the possibility that sonication might increase the transforming activity of uninfected cell DNAs by dissociating potential transforming genes from flanking sequences that might regulate their expression. The MWs of unsonicated DNAs were greater than 20×10^6 whereas those of sonicated DNA fragments were $0.3\text{--}3 \times 10^6$ (Fig. 1). Salmon sperm DNA (MW range $0.3\text{--}20 \times 10^6$, Fig. 1) and sonicated fragments of *Escherichia coli* DNA were included as controls to estimate the frequency of spontaneous transformation of the recipient cells.

DNAs were assayed by transfection of NIH 3T3 cells as previously described¹⁴, except that DNA-treated cells were transferred into soft agarose medium 7 days after transfection and colonies of transformed cells were counted after 2–3 weeks of further incubation. This procedure was found to reduce the background of spontaneous transformation of the recipient cells compared with that observed in assays of focus formation on the

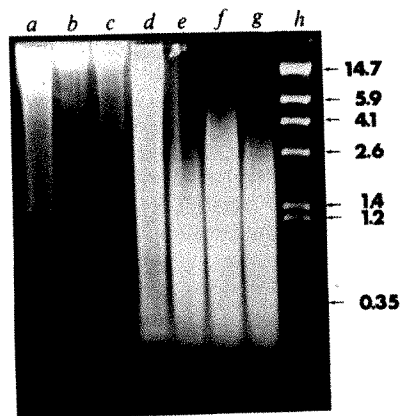


Fig. 1 Molecular weights of DNAs used in transfection assays. DNAs were extracted as described in Table 1 legend and were electrophoresed in 0.8% agarose horizontal slab gels in Tris-acetate buffer, stained with ethidium bromide and photographed under UV light¹⁴. High-MW DNAs of chicken embryo fibroblasts, NIH 3T3 cells and MC5-5 cells were electrophoresed in lanes a–c, respectively. Salmon sperm DNA was electrophoresed in lane d. DNAs of chicken embryo fibroblasts, BALB 3T3 cells and MC5-5 cells were sonicated as described in Table 1 legend and were electrophoresed in lanes e–g, respectively. The MWs of marker fragments of *Hind*III-digested λ DNA (lane h) are indicated ($\times 10^6$).

Table 1 Transformation by uninfected cell DNAs

Donor DNA	Fraction positive cultures		Transformants per μg DNA (total)
	Individual	Total	
Controls			
Salmon sperm (MW $0.3\text{--}20 \times 10^6$)	3/396	3/513	3×10^{-4}
Sonicated <i>E. coli</i> (MW $0.3\text{--}3 \times 10^6$)	0/117		
High MW ($>20 \times 10^6$)			
QT-6	1/48		
MC5-5	0/48		
CEF*	0/46	1/182	3×10^{-4}
NIH 3T3	0/40		
Sonicated fragments (MW $0.3\text{--}3 \times 10^6$)			
QT-6	5/70		
MC5-5	3/48		
CEF*	3/56	16/294	3×10^{-3}
NIH 3T3	3/80		
BALB 3T3	2/40		

High MW DNAs of avian and mammalian cells were extracted essentially as previously described²². Cells were lysed with 0.5% SDS and extracts were deproteinised by digestion with pronase, extraction with phenol and extraction with chloroform-isoamyl alcohol. DNAs were precipitated with ethanol, dissolved in SSC (0.15 M NaCl–0.015 M sodium citrate, pH 7.0), digested with RNase A, re-digested with pronase, extracted with phenol, extracted with chloroform-isoamyl alcohol, precipitated with ethanol and dissolved in SSC. *E. coli* DNA was extracted by the same procedure after lysis of the cells by treatment with lysozyme. Salmon sperm DNA (Sigma) was dissolved in SSC, extracted with phenol, extracted with chloroform-isoamyl alcohol, precipitated with ethanol and dissolved in SSC. Fragments of DNA were prepared by sonication for 10 s using the microtip of a Branson sonifier with an output of 60 W. Recipient cultures of NIH 3T3 cells were transfected with $20 \mu\text{g}$ DNA per culture as previously described¹⁴. DNA-treated cells were transferred into semi-solid medium containing 0.25% agarose¹⁴ 7 days after exposure to DNA. Colonies of transformed cells were counted after 2–3 weeks of further incubation. Data are presented as the fraction of recipient cultures that contained transformed cell colonies (generally 1–5 colonies per positive culture). Each positive culture was considered an independent event for calculation of the transformation frequency.

* CEF DNA was extracted from normal chicken embryo fibroblasts after four to six passages in culture.

original DNA-treated plates. Control experiments indicated that the number of colonies induced by DNA of avian sarcoma virus-infected cells in this assay was similar to the number of foci induced in parallel cultures that were maintained in liquid medium (0.1–1 transforming units per μg DNA).

Results of transfection assays of uninfected cell DNAs are summarised in Table 1. Transfection by high MW DNAs of uninfected cells did not increase the frequency of transformation of recipient cells compared with the background of spontaneous transformation in control cultures exposed to salmon sperm or sonicated *E. coli* DNAs. In contrast, transfection by sonicated fragments of DNAs of both transformed and non-transformed cells resulted in a ~ 10 -fold increase in transformation frequency. The increased transforming activity of sonicated DNA fragments of avian and mammalian cells, compared with salmon sperm and sonicated *E. coli* DNAs, was statistically significant at the $P < 0.001$ level (χ^2 test). The frequency of transformation by sonicated DNA fragments of uninfected avian and mammalian cells was similar to the frequency of transformation by subgenomic DNA fragments of avian sarcoma virus-infected cells¹⁵.

Transformation by high molecular weight DNAs of cells transformed by DNA fragments

Individual colonies of transformed cells were picked and grown to populations of $10^6\text{--}10^9$ cells for further study. These cells had morphologies typical of transformed mouse cells and grew to

high cell densities. Cells transformed by fragments of uninfected cell DNAs did not produce retrovirus particles (assayed by sedimentable DNA polymerase activity of culture fluids¹⁹) or infectious transforming virus. In addition, transforming virus could not be rescued from these cells by superinfection with non-transforming Moloney murine leukaemia virus.

Previous studies indicated that high MW DNAs extracted from nine spontaneous transformants of NIH 3T3 cells did not induce transformation of NIH 3T3 cells in secondary transfection assays (<0.001 transforming units per μg DNA)¹⁴. In contrast, high MW DNAs of cells transformed by subgenomic fragments of avian sarcoma virus DNA induced transformation of NIH 3T3 cells with efficiencies of ~ 0.5 transforming units per μg DNA¹⁵. We therefore assayed the transforming activity of high MW DNAs of NIH 3T3 cells that were transformed by fragments of uninfected cell DNAs to determine whether DNAs of these cells were similarly capable of inducing transformation with high efficiencies.

High MW DNAs of NIH 3T3 cells and of spontaneously transformed NIH 3T3 cells isolated after exposure to salmon sperm DNA did not induce transformation in secondary transfection assays (Table 2). In contrast, high MW DNAs of NIH 3T3 cells transformed by sonicated fragments of DNAs of QT-6 cells, MC5-5 cells, NIH 3T3 cells and normal chicken embryo fibroblasts induced efficient transformation of recipient NIH

Table 2 Transformation by DNAs of NIH 3T3 cells transformed by fragments of uninfected cell DNAs

Donor DNA	Foci per μg DNA	Colonies per μg DNA
Salmon sperm	<0.002	<0.002
NIH 3T3	<0.002	<0.002
NIH (salmon sperm DNA)	<0.005	<0.002
NIH(QT-6 DNA)	0.09	0.3
NIH(MC5-5 DNA)cl 1	0.16	1.5
NIH(MC5-5 DNA)cl 2	0.15	0.3
NIH(MC5-5 DNA)cl 3	0.08	0.2
NIH(NIH DNA)	ND	0.1
NIH(CEF DNA)cl 1	0.03	0.2
NIH(CEF DNA)cl 2	ND	0.8
NIH(CEF DNA)cl 3	ND	1.1

Salmon sperm DNA, high MW DNA of non-transformed NIH 3T3 cells and high MW DNAs of transformed NIH 3T3 cells isolated after exposure to salmon sperm DNA [NIH (salmon sperm DNA)] and to sonicated DNAs of QT-6 cells [NIH(QT-6 DNA)], MC5-5 cells (three independent isolates) [NIH(MC5-5 DNA)cl 1,2,3], NIH 3T3 cells [NIH(NIH DNA)] and normal chicken embryo fibroblasts (three independent isolates) [NIH(CEF DNA)cl 1,2,3] in the experiments described in Table 1 were assayed by transfection of NIH 3T3 cells. Some cultures were maintained under liquid medium and foci of transformed cells were counted 14–17 days after transfection. Other recipient cultures were transferred into soft agarose medium 7 days after transfection and colonies of transformed cells were counted after 2–3 weeks of further incubation. ND, not done.

3T3 cells as assayed by either focus formation or colony formation in soft agarose (Table 2). The morphologies of representative foci of transformed cells are illustrated in Fig. 2. The efficiencies of transformation by DNAs of cells transformed by DNA fragments were ~ 100 -fold higher than the original efficiencies of transformation by sonicated DNA fragments. Thus, NIH 3T3 cells transformed by DNA fragments of uninfected avian and mammalian cells apparently contained transforming genes that were transmitted to new recipient cells by transfection of high MW DNA with relatively high efficiencies.

The transforming activity of restriction endonuclease-digested DNAs of three independent clones of NIH 3T3 cells transformed by fragments of chicken embryo fibroblast DNA was investigated in experiments presented in Table 3. The transforming activity of DNA of NIH(CEF DNA) clone 1 cells was abolished by digestion with *Hind*III but not by digestion with *Eco*RI or *Bam*HI. In contrast, the transforming activities of DNAs of NIH(CEF DNA) clone 2 and clone 3 cells were abolished by digestion with *Bam*HI but not by digestion with *Eco*RI or *Hind*III. These results indicated that transformation in secondary transfection assays was mediated by specific segments of donor DNA. In addition, it seemed that the transforming activity of NIH(CEF DNA) clone 1 cells differed from the transforming activities of NIH(CEF DNA) clone 2 and clone 3 cells, although no conclusion can be drawn concerning the relationship between the transforming activities of NIH(CEF DNA) clone 2 and clone 3 cells.

Endogenous transforming genes of uninfected cells

The findings that DNA fragments of uninfected normal and chemically transformed cells induce transformation with low efficiencies and that high MW DNAs of cells transformed by DNA fragments induce transformation with high efficiencies indicate that endogenous transforming genes of uninfected cells can be activated and efficiently transmitted by transfection. These observations are consistent with the hypothesis that normal cells contain genes that are capable of inducing transformation if expressed at abnormally high levels. A model which accounts for our results in terms of this hypothesis is presented in Fig. 3. The low efficiency of transformation by sonicated DNA fragments of uninfected cells could correspond to the probability of integration of endogenous transforming genes derived

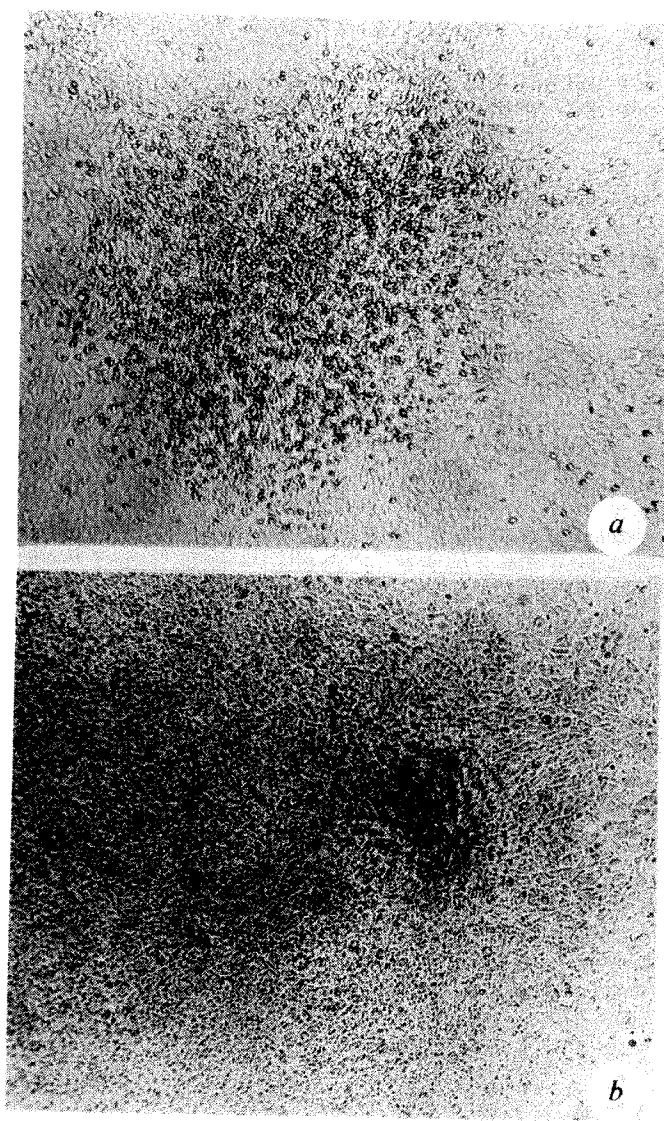


Fig. 2 Morphologies of transformed cells. Representative foci of NIH 3T3 cells transformed by DNAs of NIH(QT-6 DNA) cells (a) and of NIH(CEF DNA)cl 1 cells (b) as described in Table 2 legend were photographed with a magnification of $\times 38$.

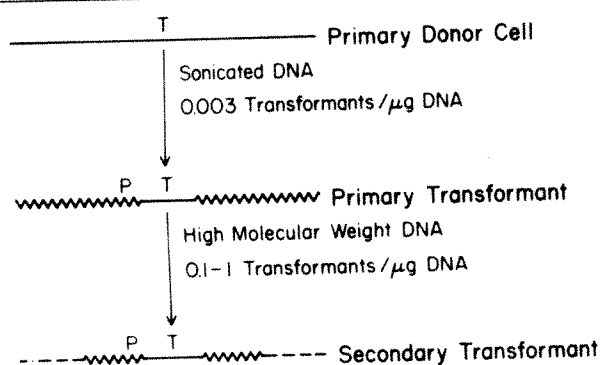


Fig. 3 Model for transfection by endogenous transforming genes. Uninfected cells used as donors of DNA contain genes that are potentially capable of inducing transformation if expressed at abnormally high levels (endogenous transforming genes). Transformation by sonicated DNAs results from integration of endogenous transforming genes (T) in the vicinity of a promoter site (P) in recipient cell DNA, resulting in abnormally high expression of the donor gene. Efficient transformation by high MW DNAs of the primary transformed cells results from transfection of secondary recipient cells by DNAs containing promoter regions (P) derived from the primary recipient genome in tandem with transforming genes (T) derived from the original donor DNA.

from the donor DNA in the vicinity of a transcriptionally active promoter in the recipient cell genome, thereby permitting abnormally high expression of the donor transforming gene. Note that the efficiency of transformation by sonicated fragments of uninfected cell DNAs (3×10^{-3} transformants per μg DNA) is similar to that by subgenomic fragments of avian sarcoma virus-infected cell DNAs ($\sim 1 \times 10^{-2}$ transformants per μg DNA)¹⁵. The transforming activity of sonicated DNA fragments, but not high MW DNAs, may indicate that the biologically active DNA fragments do not include flanking sequences that might regulate the expression of endogenous transforming genes in normal cells. The high efficiency of transformation by high MW DNAs of cells transformed by DNA fragments is consistent with transformation of the secondary recipient cells by DNAs containing promoter regions derived from the primary recipient cell genome in tandem with transforming genes derived from the original DNA fragments. Similar high efficiencies of transformation are observed in transfection assays of DNAs of cells transformed by subgenomic fragments of avian sarcoma virus DNA¹⁵ and by fragments of herpes simplex virus DNA containing the viral thymidine kinase gene²⁰. The transforming activity of avian cell DNAs in NIH 3T3 mouse cells is consistent with the transforming activity of DNAs of avian sarcoma¹⁴ and acute leukaemia¹⁵ viruses in these cells and with the biological activity of the chicken thymidine kinase gene in transfection of Ltk⁻ mouse cells²⁰.

An alternative possibility is that transformation by sonicated DNA fragments results from mutagenic activation of endo-

genous transforming genes of the recipient cells as a consequence of integration of donor DNA fragments into regulatory sequences that normally control expression of these genes. In this case also, the high efficiency of transformation by high MW DNAs of cells transformed by DNA fragments indicates that these cells contain transmissible activated endogenous transforming genes.

We are now investigating the possible relationships between the endogenous transforming genes identified here and the transforming genes of retroviruses. Molecular cloning of the transforming genes of cells transformed by fragments of uninfected cell DNAs should permit studies of these endogenous transforming genes at the molecular level and facilitate investigations of the biological activities of these genes in normal and transformed cells.

Implications for chemical carcinogenesis

The efficiency of transformation by high MW or sonicated DNAs of the two methylcholanthrene-transformed cell lines (QT-6 and MC5-5 cells) used as DNA donors in the present experiments did not differ from the efficiency of transformation by DNAs of non-transformed mouse cell lines (BALB 3T3 and NIH 3T3) or by DNAs of normal chicken embryo fibroblasts. Therefore, it seems that the two chemically transformed cell lines studied did not contain transforming genes that were efficiently transmitted by transfection of high MW DNA. These results suggest that the chemically induced transformation events that resulted in establishment of the QT-6 and MC5-5 cell lines were not transmissible by transfection. Mutational inactivation of a gene encoding a *trans*-acting regulator of the expression of an endogenous transforming gene may be an example of such a transforming lesion. Analysis of the transforming activity of DNAs of a variety of spontaneous and chemically induced neoplasms will be required to determine whether some tumours contain transforming genes that are transmissible with high efficiencies by transfection, as might result from mutational alteration of *cis*-acting regulatory sequences that control expression of endogenous transforming genes.

Since submission of this manuscript, Shih *et al.*²¹ have reported transformation of NIH 3T3 cells by high MW DNAs of 5 out of 15 chemically transformed mouse cell lines.

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Table 3 Restriction endonuclease digestion of DNAs of transformed NIH 3T3 cells

Donor DNA	Colonies per μg DNA			
	Undigested	EcoRI	HindIII	BamHI
NIH(CEF DNA)cl 1	0.3	0.5	<0.03	0.5
NIH(CEF DNA)cl 2	0.8	0.3	0.3	<0.03
NIH(CEF DNA)cl 3	1.1	0.9	0.5	<0.03

DNAs of three independent clones of NIH 3T3 cells transformed by sonicated fragments of uninfected chicken embryo fibroblast DNA were digested to completion with *EcoRI*, *HindIII* and *BamHI*. The extent of digestion was determined by inclusion of λ DNA in an aliquot of each reaction mixture and analysis of the cleavage products by electrophoresis in agarose gels¹⁴. Transforming activity of undigested and digested DNAs was assayed by transfection of NIH 3T3 cells. Colonies of transformed cells in soft agarose medium were counted as described in Table 2 legend.

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Gene transfer in intact animals

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Resistance to methotrexate was induced in bone marrow cells of mice by transformation in vitro with DNA from a drug-resistant cell line. Transformed cells were injected in vivo and haematopoietic cells expressing resistance were selected by drug treatment of recipients. Transformed cells had elevated levels of dihydrofolate reductase and demonstrated a proliferative advantage over untransformed cells, indicating successful gene transfer.

THE classic studies of Avery, Macleod and McCarty¹ described alterations of the characteristics of one strain of *Pneumococcus* by the introduction of DNA from a second strain. This formed the basis for the identification of DNA as the primary biological material for storage of genetic information, and demonstrated the feasibility of introducing new genetic information into prokaryotic organisms. Subsequent studies examined the possibility of introducing DNA into mammalian cells. This process of gene insertion is known as transformation. There has been success in a variety of tissue culture systems using a multiplicity of techniques for inserting DNA, for example, viral vectors², cell-cell fusion, fusion to cells of a limited number of chromosomes enveloped in nuclear membranes^{3,4}, and cellular endocytosis of microprecipitates of calcium-DNA complex⁴⁻⁷. Fusion techniques give a relatively high efficiency of information insertion, whereas the calcium precipitation method allows for great selectivity in the introduction of a particular DNA species.

Many mammalian tissue culture cells will take up new DNA. However, the new genetic information must confer some advantage to the cell to demonstrate that they have transformed for a particular characteristic. For example, cell lines lacking thymidine kinase (tk^-) are readily transformed by appropriate DNA to a tk^+ status when grown in the presence of a folic acid inhibitor and thymidine^{6,7}. The presumed mechanism is the integration and functional expression of DNA specifying thymidine kinase by perhaps one cell in 10^6 . This tk^+ cell has a proliferative advantage over tk^- cells when *de novo* DNA synthesis is blocked and the cells are dependent on exogenous thymidine for DNA synthesis and proliferation.

The concept of transferring new genetic information and selecting for its expression using drug-resistance genes and drug

treatment can in principle be applied to intact animals. Certain limitations to such genetic manipulation would seem obvious. The drug used to apply selective pressure must have an acceptable level of toxicity. It must affect the particular tissue being transformed to drug resistance. Finally, if adult animals are used, only tissues demonstrating persistent proliferation throughout life would be amenable to drug-selective pressures. Anti-metabolite drugs used in cancer chemotherapy and blood-forming tissues satisfy these requirements and seem to be ideally suited for such genetic manipulations.

We describe here insertion and selection for genes conferring resistance to methotrexate in bone marrow cells of intact mice. Methotrexate (MTX) is an anti-cancer drug that acts as a folic acid antagonist by inhibiting the enzyme dihydrofolate reductase (DHFR)⁸. Mouse DNA enriched for sequences coding for dihydrofolate reductase was used to transform bone marrow cells *in vitro* and MTX was used as the selective agent *in vivo*. A well studied chromosomal marker was used to distinguish cells transformed to drug resistance from the normal cells of the recipient or from mock-transformed cells introduced as a control.

Strategy of selection of transformed cells

Mouse bone marrow cells with a distinctive chromosomal marker (T6T6)⁹ were obtained from intact animals and treated *in vitro* with a calcium microprecipitate of DNA^{6,7} from a mouse cell line selected for high resistance to MTX and containing reiterated sequences of genes coding for DHFR^{10,11}. The treated marrow, presumed to contain a few stem cells transformed to MTX resistance, was mixed in equal proportions with

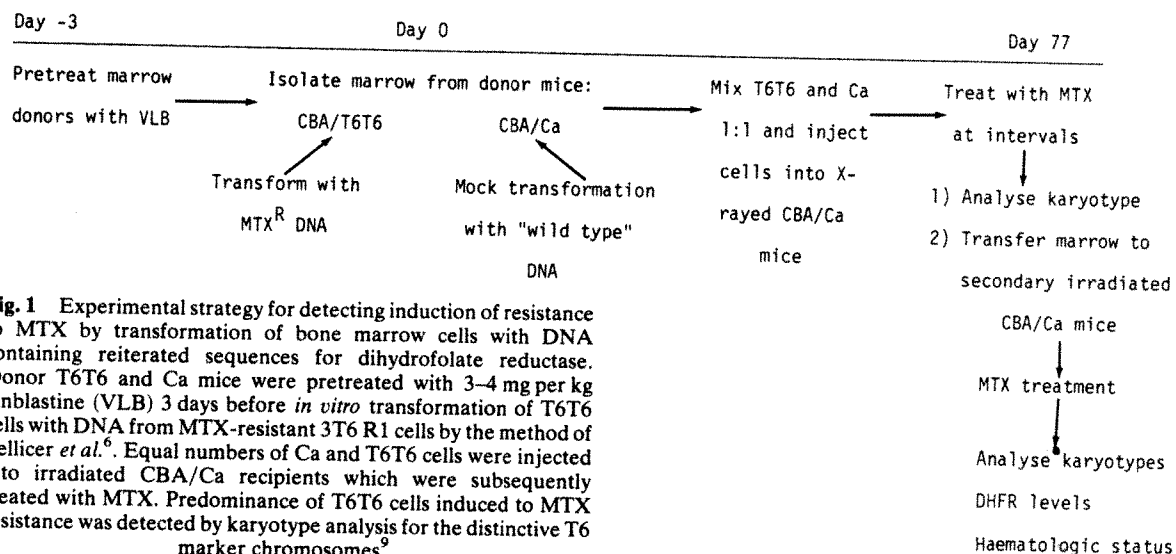


Fig. 1 Experimental strategy for detecting induction of resistance to MTX by transformation of bone marrow cells with DNA containing reiterated sequences for dihydrofolate reductase. Donor T6T6 and Ca mice were pretreated with 3–4 mg per kg vinblastine (VLB) 3 days before *in vitro* transformation of T6T6 cells with DNA from MTX-resistant 3T6 R1 cells by the method of Pellicer *et al.*⁶. Equal numbers of Ca and T6T6 cells were injected into irradiated CBA/Ca recipients which were subsequently treated with MTX. Predominance of T6T6 cells induced to MTX resistance was detected by karyotype analysis for the distinctive T6 marker chromosomes⁹.

Table 1 Karyotype analysis of marrow cells of irradiated CBA/Ca mice receiving a 1:1 mixture of control Ca and transformed T6T6 marrow cells

Recipient*	Duration of MTX treatment (days)	Karyotype (% T6T6)
Primary 1	0-24	57
Primary 2	0-32	59
Secondary 2	32-46	79
Primary 3	0-39	67
Secondary 3a	39-53	97
Secondary 3b	39-67	93
Secondary 3c	39-73	84

*Irradiated CBA/Ca recipients of the 1:1 mixture of Ca cells transformed with wild-type DNA and T6T6 cells transformed with 3T6 R1 DNA were designated 'primary' and each mouse was given a unique number. The day of infusion is designated '0'. Recipients of marrow from 'primary' animals are designated 'secondary' and bear the same identifying number. Karyotype analyses of recipient bone marrow cells were performed after the designated interval of MTX treatment. Between 50 and 100 chromosome spreads were analysed.

Table 2 Karyotype analysis of marrow cells of CBA/Ca mice receiving a 1:1 mixture of control Ca and transformed T6 marrow cells

Recipient*	Period with MTX (days)	Period without MTX (days)	Karyotype (% T6)
Primary 1	0-33	—	79
Primary 2	0-40	—	75
Primary 3	0-47	—	74
Primary 3	0-47	48-68	83
Secondary 3	47-61	—	88, 88, 100†
Primary 4	0-54	—	75
Secondary 4	54-72	—	83
Primary 5	0-65	—	96
Primary 5	0-65	66-113	63

*As in Table 1.

†Three secondary recipients.

syngeneic marrow cells lacking the chromosomal marker (Ca). These cells had been incubated in transforming conditions with control DNA lacking MTX-resistance sequences. The cell mixture was injected into genetically compatible animals that had been irradiated to reduce endogenous haematopoiesis. The infused cells gradually restored blood cell formation in these marrow-depleted recipients. During the period of reconstitution the animals were treated with MTX to produce a relative inhibition of untransformed stem cells and allow for a selective proliferative advantage for those transformed cells which had incorporated a functional gene specifying DHFR. Transformed cells were identified by their distinctive chromosomal marker¹². If there were no selective advantage, then the ratio of marked to unmarked dividing haematopoietic cells would be 50:50 or less because of the contribution of unmarked cells from the irradiated recipient. If, however, there were a proliferative advantage, then marked cells with increased levels of DHFR should be found to constitute more than 50% of the dividing marrow population.

In addition to this indirect means of detecting a population of haematopoietic cells transformed to MTX resistance, we have measured directly the levels of dihydrofolate reductase. Finally, we found that conventional measures of the haematopoietic status of MTX-treated animals provide a convenient measure of the success of the transformation procedure.

The T6 marker was selected because it is easily identifiable (low rate of false positive or negative scoring) and because of the

availability of completely syngeneic animals possessing and lacking this marker. CBA/H-T6T6 mice have the chromosomal anomaly whereas compatible CBA/Ca mice lack the marker.

Bone marrow of mice was selected for transformation by DNA because of its accessibility, high rate of proliferation, and the persistence of pluripotent stem cells throughout adult life. Of all the cell types present in the marrow, only transformation of appropriate stem cells should lead to the establishment of long-term MTX-resistant haematopoietic activity in recipient animals. It is therefore advantageous to choose cell populations rich in stem cells for transformation. Furthermore, transformation may be more efficient in dividing than in stationary cells. For these reasons we attempted to increase the level of proliferating stem cells by pretreating the donor animals with a mitotic inhibitor (vinblastine)¹³ before their use in gene transfer experiments.

MTX was chosen as the selective drug for several reasons: (1) the recent description of mouse cell lines containing highly reiterated gene sequences specifying DHFR, an enzyme conferring resistance to MTX^{10,11}; (2) the titratable suppressive effect of MTX on bone marrow cells; and (3) the potential applicability of the MTX system to human diseases¹⁴.

Haematopoietic effects of MTX treatment in the mouse

We first established a schedule of MTX treatment that would select for drug-resistant haematopoietic cells without lethality in control animals. Groups of normal CBA or C3H mice (18-25 g) were treated using a schedule of 0.5 mg per kg for four doses, 2 mg per kg for four doses and then 4 mg per kg three times weekly. This was not lethal but profoundly affected haematopoiesis. The haematocrit and tibial cellularity were found to be the easiest and most reliable haematological parameters to follow and remained depressed in animals treated continuously with MTX for at least 3 months. No difference in sensitivity to MTX was observed in the mouse strains CBA/Ca and CBA/H-T6T6.

Transformation of mouse bone marrow *in vitro*

The experimental strategy outlined in Fig. 1 was used as a guide for analysing the effectiveness of the transformation procedure.

Highly MTX-resistant mouse fibroblast Swiss 3T6 cells¹⁵ containing reiterated structural genes specifying DHFR were maintained in 4×10^{-4} M MTX and designated 3T6 R1. DNA was isolated from 3T6 R1 and from non-resistant (wild type) mouse cell lines including 3T6 (fibroblastic) and L1210 (lymphocytic leukaemia) and in later experiments from salmon sperm (Sigma). The relative ratio of DHFR synthesis and number of gene copies in 3T6 R1 and 3T6 was ~30:1 (ref. 15).

Table 3 Karyotype analysis of bone marrow and pluripotent stem cells from CBA/Ca mice receiving 1:1 mixture of control Ca and transformed T6T6 bone marrow cells

Recipient	Duration of MTX (days)	Bone marrow karyotype T6T6 (%)	Karyotype of spleen colonies		
			T6T6 (%)	Ca (%)	Mixed (%)
Primary 1	0-24	57	50	50	0
Primary 2	0-40	75	57	26	17
Primary 3	0-47	74	58	8	33

Individual spleen colonies were removed 10 days after inoculation of irradiated recipient with bone marrow cells. A single cell suspension was made from each colony and cells were incubated with colcemid $3 \mu\text{g ml}^{-1}$ for 90 min before treatment with hypotonic KCl and fixation with acetic acid/ethanol for chromosome spreads¹².

Table 4 Karyotype analysis of marrow cells of CBA/T6T6 mice receiving a 1:1 mixture of control T6 cells and transformed Ca marrow cells

Recipient	Expt	MTX treatment	Duration (days)	Karyotype (% Ca)
Primary 1	1	Yes	42	55
Primary 2	1	None	59	33
Primary 3	1	Yes	59	62
Primary 4	1	Yes	71	68
Primary 5	1	None	71	35
Primary 6	1	Yes	97	72
Primary 1	2	Yes	56	67
Primary 2	2	None	56	40

Recipient T6 mice received aliquots of a 1:1 mixture of T6 cells transformed with wild-type DNA and Ca cells transformed with 3T6 R1 DNA. Animals were either untreated or treated with MTX.

DNA co-precipitated with calcium phosphate was used to transform cells by the method of Bachetti and Graham⁴ as modified by Wigler *et al.*⁷.

Equal numbers of CBA/Ca and CBA/H-T6T6 mice were injected intraperitoneally with vinblastine at 3 or 4 mg per kg 3 days before marrow was removed for *in vitro* transformation. The resulting mitotic inhibition is followed by a burst of proliferation¹³. Assays of spleen colony-forming cells (CFU-S) from animals thus treated were relatively depleted of mature cells and enriched ~threefold in pluripotent CFU-S. On the day of transformation (day 0, Fig. 1) single cell suspensions in McCoy's 5A medium with 15% fetal calf serum (FCS) were obtained from femurs and tibias. Cells from Ca and T6T6 animals were placed in separate pools. Cell suspensions of 5×10^6 in 10 ml complete medium (CM) were incubated with 1.0 ml Ca-precipitated DNA containing 40 μ g DNA as described by Wigler *et al.*⁷ for 4 h at 37 °C in 5% CO₂ in tissue culture flasks. T6T6 cells were incubated with 2 or 4 μ g DNA from 3T6 RI MTX-resistant cells, and CBA/Ca marrow cells were incubated with control DNA preparations from MTX-sensitive cells. Thereafter, loosely adherent cells were collected and centrifuged at 150g for 10 min and resuspended in DNA-free CM. After careful cell counts, Ca and T6T6 cells were combined in a ratio of 1:1 and between 5×10^6 and 5×10^7 of the combined cells were injected intravenously into recipient CBA/Ca mice in 0.3–0.4 ml in McCoy's medium with FCS. These recipients had received 850 rad from a cobalt source 24 h previously to eradicate endogenous haematopoiesis. This dose had low lethality but virtually eradicated endogenous CFU-S¹⁶. Between 48 and 96 h after injection, the recipient animals began treatment with the previously established MTX protocol.

Selection of drug-resistant marrow cells

The irradiated mice receiving mixtures of control Ca cells and T6T6 cells transformed with 3T6 R1 DNA were treated with MTX for 24–77 days. Marrow samples were obtained periodically and analysed for karyotype distribution, cellularity and CFU-S content and were injected into secondary irradiated CBA/Ca recipients (Tables 1, 2). Between about days 30 and 40 a clear increase in the percentage of bone marrow cells displaying the T6T6 marker was observed in primary recipient animals. Marrow from these mice was injected into irradiated secondary recipients which were then treated with MTX. They too showed an increased ratio of T6T6 to Ca karyotypes. This same pattern was seen in five independent experiments involving 19 primary recipient animals and 30 secondary recipients. Only two experiments during this same period failed to show a predominance of transformed karyotype.

When MTX treatment of animals receiving transformed marrow cells was stopped, the predominance of T6T6 karyotypes persisted for at least 3 weeks (primary recipient 3, Table

2) but gradually diminished by 8 weeks without treatment (primary recipient 5, Table 2). In later studies the stability of the transformed karyotype in the absence of drug selection was variable (data not shown).

To analyse whether the predominance of T6T6-marked cells involved pluripotent stem cells as well as other proliferating marrow cells, marrow was taken from selected primary recipient animals and 5×10^4 cells were injected into irradiated recipient CBA/Ca mice in a typical CFU-S assay¹⁶. Ten days later the secondary recipients were killed and individual spleen colonies removed for karyotype analysis. As seen in Table 3, the T6T6 karyotype predominated in the pluripotent marrow stem cell population. Mixed T6T6–Ca spleen colonies were also seen, presumably resulting from development of T6T6 colonies on a background of endogenous haematopoiesis in the Ca animals.

Effect of drug administration on cell predominance

We performed control experiments to determine whether T6T6-marked cells had any proliferative advantage or increased resistance to MTX and to analyse the contribution of endogenous haematopoietic repopulation in irradiated CBA/Ca animals. Experimental animals receiving an equal mixture of mock transformed Ca and mock transformed T6T6 and either untreated or treated with MTX for up to 2 months had a predominance of Ca karyotypes anticipated from the contributions of infused Ca cells and endogenous Ca cells (% Ca = 68 ± 12 ; mean \pm 2s.d.).

Finally, in two independent experiments we reversed the usual procedure and transformed Ca cells and used T6 cells as the controls. After injection of a 1:1 mixture of Ca and T6 into irradiated T6 animals they were treated with MTX or left untreated for 2 months. The Ca karyotype predominated only when MTX was administered (Table 4). This gave an unambiguous demonstration that drug therapy determined the predominant marrow population and that the karyotype *per se* did not influence predominance; that is, no inherently greater resistance to MTX was associated with either the CBA/Ca or the CBA/H-T6T6 strain.

Table 5 Haematological status of mice receiving transformed bone marrow

Recipients	MTX treatment (days)	CFU-S (per 5×10^4 cells)	Tibial cellularity (cells per 10^6)	Haematocrit (%)
Controls: saline-treated (15)	None	11.3 (5–15)	8.7 (7.1–10.6)	42 (38–47)
Controls: MTX-treated (15)	21–77	—	3.9 (3.3–4.9)	26 (23–39)
Primary 1	0–33	8.8 (7–9)	13.9	—
Primary 2	0–40	16 (13–19)	9.6	—
Primary 3	0–47	22 (18–25)	6.0	—
Primary 4	0–54	—	9.6	—
Secondary 4	54–72	—	6.6	40
Primary 5	0–65	—	8.2	47

Control animals received no irradiation or bone marrow cells and were injected with saline or MTX as described. Number of animals is indicated in parentheses in column Recipients. Numbers in parentheses in other columns indicate the range of values observed. Primary recipient CBA/Ca mice were irradiated (850 rad) and received a 1:1 mix of control Ca and transformed T6 cells before treatment with MTX for the periods indicated. Secondary recipients were irradiated and received marrow from primary recipients. CFU-S were assayed by injecting 5×10^4 marrow cells into irradiated CBA/Ca recipient mice and counting spleen colonies 10 days later¹⁶.

Haematological status of mice receiving transformed marrow

When it became apparent that cells transformed with MTX-resistant DNA predominated in the marrow of drug-treated recipient animals, simple tests were performed to assess the animals' haematological status. Animals receiving transformed bone marrow and MTX have higher haematocrits and tibial cellularity than control animals receiving MTX but no transforming DNA (Table 5). Furthermore, they have high levels of CFU-S similar to control animals that did not receive radiation or bone marrow cells. Similar results were seen in other independent experiments. Thus, the haematological status of mice receiving transformed bone marrow treated with 3T6 R1 DNA *in vitro* returned toward normal despite persistent treatment with MTX. Autopsies were performed on these animals at intervals up to 150 days after transformation, and sections of visceral organs were taken for microscopic study. No abnormalities were revealed and the animals were clinically well at the time of death.

DHFR assay

To assess whether drug resistance was related to higher enzyme levels in mice receiving transformed bone marrow, spleens were removed from primary, secondary or tertiary recipients of transformed marrow from four independent experiments and assayed for DHFR. These recipient mice were initially treated with MTX and treatment was terminated 5–7 days before collection of tissue for the DHFR assays. Appropriate controls from 12 syngeneic animals, either irradiated or not, and untreated or treated with MTX for periods of up to 6 weeks, were also used as spleen donors. In each case, conditions for control animals were chosen to match those of the experimental group. A radiometric assay for DHFR was performed on sonicated cell-free extracts of spleen^{17,18}. DHFR-specific activity was two- to fourfold greater in the spleen extracts of animals receiving transformed bone marrow than in controls (Table 6). These were considered to be underestimates of the elevations of DHFR in the haematopoietic cells of animals receiving transformed marrow, as the extracts of spleen also included stromal and capsular tissues and non-proliferating lymphoid cells.

Discussion

We have described studies aimed at demonstrating transformation of bone marrow cells to MTX resistance by gene transfer. Extrapolation from tissue culture studies suggests an efficiency of functional gene transfer of 10^{-5} or 10^{-6} for the thymidine kinase gene^{6,7}. Mice receiving 10^7 bone marrow cells receive approximately 10^4 – 10^5 pluripotent stem cells and perhaps several hundredfold greater numbers of committed myeloid and erythroid stem cells. The infusion of an effectively transformed haematopoietic pluripotent stem cell would therefore be expected to be a relatively rare event, occurring in about 1 mouse in 10, yet after 50 or more days of drug selection, animals routinely had elevated levels of the chromosomal marker indicative of cells transformed to drug resistance. Gene insertion into haematopoietic cells may therefore be easier than in some other established cell lines.

As the DHFR genes of our MTX-resistant mouse cell line seem to be similar to those of native mouse DNA, it was not possible to demonstrate unambiguously the introduction of new genes into marrow cells by the Southern blot hybridisation technique¹⁹. Consequently, we undertook a parallel series of identical experiments using the herpes simplex virus thymidine kinase (HSVtk) gene as the transforming agent, rather than DNA derived from a MTX-resistant mouse cell line. With HSVtk DNA used for marrow cell transformation, it has been possible to demonstrate virus-specific gene sequences in mouse spleen cells²⁰. Consequently, several types of drug-resistance genes may be useful in transformation studies in intact animals.

Table 6 Dihydrofolate reductase activity in spleen and karyotype analysis of bone marrow in CBA/Ca recipients receiving 1:1 mixture of control Ca and transformed T6T6 bone marrow cells

Recipient	Duration of MTX (days)	Bone marrow karyotype predominance (%)	DHFR specific activity (% control)
Primary	0–42	49	168
Primary	0–59	62*	375
Secondary	39–67	93	391
Secondary	54–72	83	183
Secondary	65–107	—	208
Tertiary	53–80	—	224

Spleen fragments were sonicated for 30 s in the cold and assayed for DHFR by the method of Hayman *et al.*¹⁸. Results are expressed as the per cent of DHFR in spleens of simultaneously killed control mice.

*This animal received transformed Ca cells and had Ca karyotype predominance. All others received transformed T6T6 cells.

The transfer of genes for drug resistance to haematopoietic cells *in vitro* and their selection in intact animals *in vivo* suggest a variety of clinical applications, for example: (1) the transfer of drug-resistance genes with the objective of enabling patients with cancer to tolerate higher doses of anti-neoplastic drugs; and (2) insertion of drug-resistance genes coupled to other genes to treat human genetic diseases such as the haemoglobinopathies.

Suppression of bone marrow haematopoiesis is one of the main limitations to intensive treatment with most clinical anticancer drugs. If the resistance of marrow cells could be enhanced by simple procedures, then higher doses of drugs could be used to treat cancers of other tissues. This might circumvent dose-limiting marrow-suppressive toxicity and allow better control of a variety of solid tumours. These studies indicate that mice receiving marrow transformed with MTX-resistant DNA tolerate high doses of MTX for long periods of time with nearly normal haematological parameters.

The haemoglobinopathies such as sickle cell disease and thalassaemia seem to be natural targets for treatment by gene transfer techniques. Insertion of a normally regulated and structurally normal β -globin gene should in theory correct the defect in β -thalassaemia major and sickle cell disease. However, as a normal β -globin gene should not itself confer any proliferative advantage to transformed marrow cells, it would be necessary to link it in some way to a gene whose expression is susceptible to selective pressures. Drug-resistance genes are natural candidates for this role.

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Unequal crossing over in the ribosomal DNA of *Saccharomyces cerevisiae*

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Unequal sister chromatid exchanges occur at the ribosomal DNA locus of yeast during mitotic growth. The frequency of unequal crossing over, as measured by the deletion or duplication of an inserted genetic marker (LEU2), is sufficient to maintain the sequence homogeneity of the rDNA repeat units.

THE ribosomal RNA genes of yeast, *Drosophila*, *Xenopus*, silkworm and sea urchin are arranged in clusters of tandemly arrayed repeating units¹⁻⁸. Yeast rDNA consists of approximately 140 copies of a 9-kilobase repeat unit⁹. Each repeat unit codes for a 5S RNA, and for a 35S precursor RNA which is subsequently degraded to mature 25S, 18S and 5.8S ribosomal RNAs^{1,2,10,11}. Many restriction endonuclease sites have been mapped on the rDNA repeat unit^{1,2,12} and restriction analysis has shown that the repeat units are homogeneous and tandemly arranged. Restriction fragments greater than one repeat unit in length have been cloned^{2,13}.

The histone genes of sea urchin, *Drosophila* and chicken¹⁴⁻¹⁷, as well as other highly repeated sequences and satellite DNAs^{18,19} have also been shown to occur in direct tandem arrays. Repeat units in related strains or species show considerable sequence divergence; yet within any one strain the repeat units are quite homogeneous. Variation between repeat units within one cluster has been detected in *Drosophila* rDNA^{3,4} where some repeat units contain large insertions, and in *Xenopus* where length heterogeneity is due to variation in the copy number of a small internal repeat⁵. No repeat unit heterogeneity has ever been detected in the rDNA of *Saccharomyces cerevisiae*. Thus, sequence variation that is introduced by mutation in individual repeat units must be fixed throughout the entire cluster of repeats. Several mechanisms have been proposed for this process of horizontal evolution or rectification: master slave correction²⁰, democratic gene conversion²¹, saltatory replication²², and unequal crossing over^{23,24}. However, no direct experimental test of these mechanisms has been reported. This is partly due to the difficulty of classical genetic analysis with repeated genes.

We have recently reported the construction of yeast strains with insertions at the rDNA locus²⁵. A plasmid containing yeast rDNA and the *LEU2* gene was made *in vitro* and introduced into yeast by transformation (see Fig. 1). Plasmid integration occurs by homologous recombination; the large amount of homologous rDNA results in preferential integration of the plasmid at the rDNA locus. The transformants thus have a functional copy of the *LEU2* gene in the rDNA cluster which is easily scored in a *leu*⁻ chromosomal background. We have used this marker to detect the deletions and duplications that are generated by unequal crossing over (see Fig. 2). Unequal crossing over occurs during mitotic growth of haploid strains, and causes deletions and duplications of six to eight repeat units. The size of the duplications was measured by restriction enzyme analysis. The relatively small size of the duplications indicates that sister chromatid pairing in the rDNA region is an ordered process. Computer projections from the measured frequency of unequal crossing over show that this process is sufficient to explain the sequence homogeneity of the rDNA repeat units of yeast.

Insertion of *LEU2* and pBR322 at the rDNA locus

The construction of the hybrid plasmid pSZ20 has been described²⁵. The plasmid consists of two fragments of yeast DNA inserted into restriction sites on the *Escherichia coli* plasmid vector pBR322 (ref. 26). The 4.4 kilobase *Bgl*II-B fragment of rDNA is inserted at the *Bam*H-I site of the vector, and serves to direct plasmid integration into the rDNA locus. A 3.5-kilobase *Sall*-*Xho*I fragment containing the intact *LEU2* gene^{25,27,28} is inserted at the *Sall* site of the vector. This fragment complements a non-reverting double mutation in the chromosomal *leu2* gene, and is used for the selection of transformants^{29,30}.

Plasmid pSZ20 DNA transforms yeast with high efficiency and yields unstable transformants. This is apparently due to an origin of DNA replication on the rDNA fragment in pSZ20 (refs 25, 31) which allows autonomous replication⁴² of the free plasmid in yeast. The transformation frequency is high because chromosomal integration is not required for plasmid maintenance; the transformants are unstable due to random segregation of the plasmid molecules during mitosis. Stable integrated-plasmid derivatives are readily obtained by subcloning the initial transformants. The large amount of chromosomal

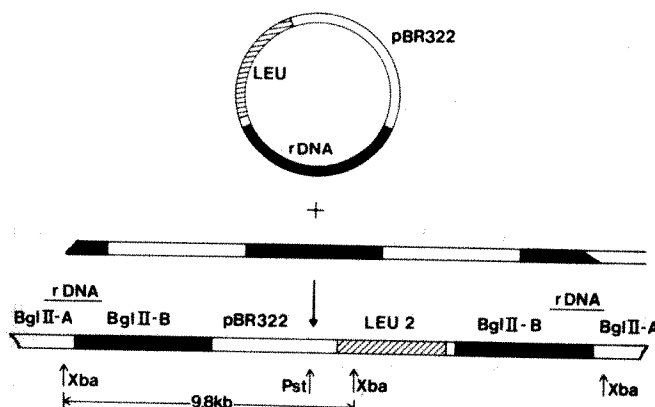


Fig. 1 Insertion of *LEU2* and pBR322 into the yeast rDNA locus. The circular plasmid pSZ20 was constructed by ligation of restriction fragments of yeast rDNA, the yeast *LEU2* gene, and the *E. coli* vector pBR322 (see ref. 25 for details of construction). The rDNA in the plasmid is a 4.4 kilobase *Bgl*II-B fragment that is approximately half of the rDNA repeat unit. Recombination with chromosomal rDNA homologous to this fragment results in plasmid integration. The integrated structure consists of *LEU2* and pBR322 sequences flanked by a direct duplication of the rDNA *Bgl*II-B fragment. Digestion of this DNA with *Xba*I generates a 9.8 kilobase fragment containing the pBR322 sequences. *Pst*I cuts near one end of the pBR322 DNA, but does not cut rDNA; thus the pBR322 remains attached to long undigested rDNA fragments unless another copy of the insertion is nearby.

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rDNA homologous to the plasmid rDNA results in a high rate of plasmid integration. The integrated-plasmid transformants are sufficiently stable through both mitosis and meiosis that the inserted marker can be genetically manipulated by standard methods.

DNA prepared from independent stable transformants was tested for the presence of restriction fragments diagnostic of the site of plasmid integration. Integration of one to four copies of the plasmid occurred at the rDNA locus in all strains tested²⁵, with multiple copies always occurring in tandem arrays. We have never observed free plasmid or plasmid integrated at other loci. Transformants containing a single copy of plasmid were most abundant (about 50%).

Restriction sites on the circular plasmid pSZ20, the chromosomal rDNA, and the integrated structure are shown in Fig. 1. The fragments generated by *Xba*I and *Pst*I are relevant to the subsequent analysis of unequal crossing over. *Xba*I generates a 9.8-kilobase fragment containing pBR322 sequences. This fragment was used for copy number measurements in which the intensity of this band was compared to the intensity of an independent single copy control. The enzyme *Pst*I cuts the plasmid once, near one end of the pBR322 DNA, without cutting rDNA at all. Therefore *Pst*I digestion of a strain with either a single or two widely separated copies of the insertion does not generate a discrete fragment carrying pBR322 DNA; the pBR322 is attached to a long undigested stretch of rDNA. Conversely, if two copies of the insertion are close together, a discrete *Pst*I fragment will be generated which spans the duplication (see Fig. 2).

Deletion of the *LEU2*-pBR322 insertion

An integrated copy of pSZ20 may be thought of as an insertion mutation. Any rectification mechanisms which operate within the rDNA cluster will tend to either eliminate or duplicate this mutation. The only functional copy of the *LEU2* gene in strain T16 is that inserted in the rDNA locus. The rate at which the insertion was spontaneously deleted was measured by a fluctuation test³² as follows. The insertion strain T16 was grown in complete medium (YPD) to stationary phase, diluted, and distributed into the wells of microtitre dishes so that about one-fourth of the wells would contain a cell. The dishes were incubated at 30 °C for 12 h to allow for six to eight generations of growth. The contents of each well were then mixed and plated onto a YPD plate. After growth at 30 °C for 2 days, the plates with colonies were replicated onto synthetic media without leucine, and scored the next day for the presence or absence of *leu*⁻ colonies.

Of 298 YPD plates, 69 (24%) had colonies. Therefore, most of the wells used to inoculate these plates started with one cell. The 67 plates scored had an average of 140 colonies per plate (9,415 total). Nine of these plates had one or more *leu*⁻ colonies, indicating that a deletion event had occurred during growth of the initial cell in the microtitre well. Each of these nine plates represents an independent deletion event. Therefore, nine events occurred during the growth of 9,415 cells, and the frequency of deletion per generation is $9/(9,415 \times 2)$, or approximately 5×10^{-4} per generation.

Deletion of the inserted *LEU2* gene was stimulated approximately 10-fold by UV irradiation to 50% survival. This observation made it much easier to pick well defined sector colonies. For this reason the analysis of unequal crossing over was done first with UV-induced sector colonies, and then repeated with spontaneously arising sectors.

Duplications generated by unequal crossing over

Unequal cross-overs can result in deletion of an insertion from one chromatid and duplication of the insertion in the other (Fig. 2). These sister chromatids are distributed to the two daughter cells after mitosis and result in a sector colony with two copies of the insertion in the *LEU*⁺ side. In contrast, a non-reciprocal

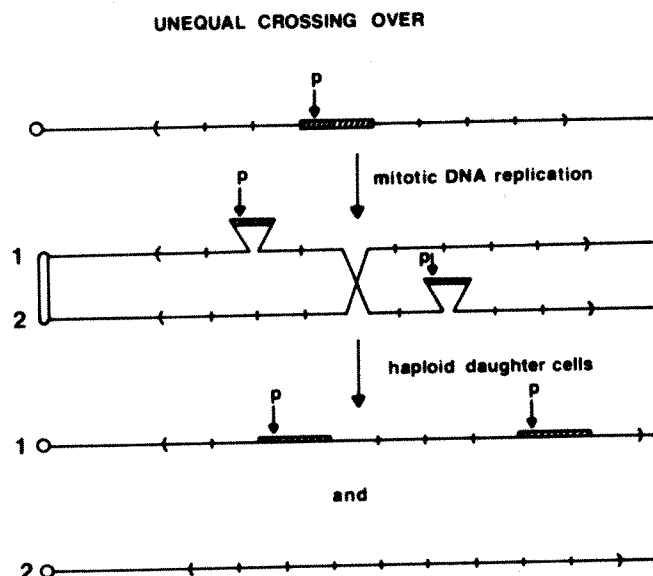


Fig. 2 Unequal crossing over can lead to duplication or deletion of the rDNA insertion. The parental strain (top) carries a single copy of the insertion, represented as a shaded box embedded in a series of tandemly arrayed rDNA repeats. Following mitotic DNA replication, the two sister chromatids pair prior to recombination. Pairing out of register leads to displacement of the two copies of the insertion relative to each other. Recombination between the displaced copies leads to one daughter cell having two copies and one having none. The daughter cells grow up side by side and form a sector colony in which cells derived from the deletion event are *leu*⁻. The event is easily detected by replica plating the colonies to media lacking leucine.

event such as looping-out excision would leave the *LEU*⁺ side of the sector with only one copy of the insertion.

The *leu*⁻ sides of 10 UV-induced sectors and 11 spontaneous sectors were tested for deletion of the insertion by colony hybridisation³⁰ with labelled pBR322 DNA. A strong hybridisation signal was obtained with the original insertion strain; none was detected with any of the *leu*⁻ strains from sector colonies or with the non-transformed parental strain (data not shown). Thus, the sector colonies result from events causing deletion of the inserted *LEU2* gene.

Duplications of the insertion in the *LEU*⁺ side of each sector were detected as follows. DNA was prepared from each strain³³ and digested with the restriction endonuclease *Xba*I to generate an identical fragment carrying the pBR322 DNA from each insertion. The DNA fragments were resolved by agarose gel electrophoresis and transferred to nitrocellulose paper by the filter blotting method³⁴. The filter was hybridised with a labelled probe containing pBR322 sequences for detection of the insertion, and *SUP3* sequences for detection of the *Xba*I *SUP3* fragment. The *SUP3* fragment serves as an independent single copy internal standard. With this method it appeared that four of the UV-induced sectors (S1, 3, 7, 9) and at least six of the spontaneous sectors (S12, 14, 15, 18, 20, 21 and perhaps S16 and 17) had a higher copy number for the insertion than the parental strain. Variation in the efficiency of DNA transfer to the filter made it difficult to obtain quantitative evidence for duplications with this method. This problem was avoided with the qualitative procedure described below.

Digestion of DNA from duplication strains with the restriction enzyme *Pst*I generates a discrete fragment carrying pBR322 sequences. In the absence of a duplication the pBR322 sequences remain attached to the rDNA, which is not digested by *Pst*I and is larger than the shear size of the isolated DNA. The detection of a discrete *Pst*I fragment is therefore strong evidence for a duplication. In addition, the size of this fragment is a measure of the size of the deletions and duplications generated by unequal crossing over. DNA from strains of all the *LEU*⁺

sectors was digested with *Pst*I; the fragments were then separated by electrophoresis through a low percentage agarose gel. In this system large fragments are well resolved and can be measured accurately. The DNA was transferred to nitrocellulose paper as above and hybridised with labelled pBR322 DNA. Hybridisation was detected with fragments of a length equal to the size of the insertion plus an integral number of rDNA repeat units (see Figs 3 and 4). Four of the ten UV-induced sectors (S1, 3, 7, 9) had duplications, ranging from 1 to 4 repeat units in length. Of the seven spontaneous sectors tested, six (S14, 15, 17, 18, 20, 21) had duplications while one did not (S19). The duplications were of 6, 7 or 8 repeat units. The data for the four UV induced and three of the spontaneous duplications are shown in Figs 3 and 4. All of the strains shown to have duplications by this method had an increased copy number of the insertion (data not shown).

The significance of the difference between UV induced and spontaneous duplications is not clear. In all cases the duplications consist of a small number of rDNA repeats (1 to 8) relative to the size of the rDNA cluster (140 repeats). This distribution could not result from a random pairing of the rDNA repeat units, and must therefore reflect an ordered pairing of the sister chromatids prior to recombination. On most of the lanes, faint bands are seen in addition to one major band. This is apparently due to secondary recombination events that result in displacement of the duplicated copies relative to each other. One of the UV-induced sectors has been completely

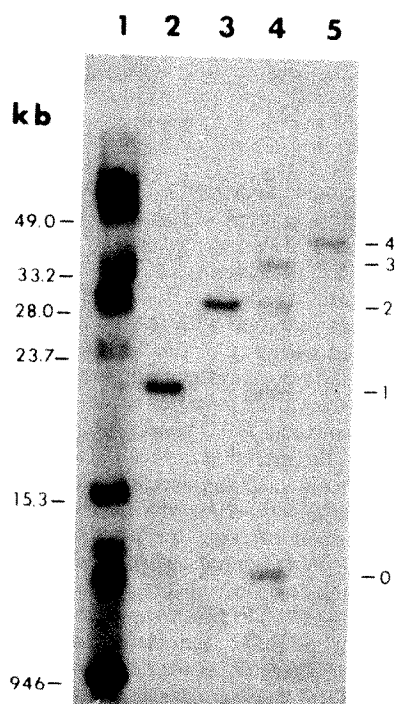


Fig. 3 Duplications produced by UV-induced unequal crossing over. DNA from the four UV-induced duplication strains was digested with *Pst*I and electrophoresed on an 0.3% agarose gel at 1 V cm^{-1} for 36 h. The molecular weight markers are a mixture of *Sa*II and *Hind*III digested fragments of λ DNA. The DNA fragments were transferred to a nitrocellulose filter and annealed with labelled pBR322 DNA. The size markers were later visualised in a separate hybridisation with labelled λ DNA. The labelled pBR322 DNA hybridised to yeast DNA fragments corresponding in size to one copy of the insertion (12.2 kilobases) plus an integral number of rDNA repeats (8.9 kilobases), as expected for a fragment spanning a duplication. Strains S1, S3 and S10 (lanes 2, 3 and 5) have fragments corresponding to duplications of 1, 2 and 4 rDNA repeat units, respectively. The pattern for S7 (lane 4) is most simply interpreted as due to randomisation by secondary recombination events, with bands that correspond to 0, 1, 2, 3 and 4 repeat unit separations of the duplicated insertion copies. In all cases faint bands are present at these positions.

randomised by such secondary events. Analysis of subclones from this culture showed that in individual cells the two insertion copies were separated by from one to five rDNA repeat units. In addition, some cells again showed randomised patterns (data not shown).

The rate of rDNA rectification

We have measured the frequency of spontaneous deletion of an insertion in the rDNA locus. These deletions are the result of unequal crossing over. Only cross-overs that occur between rDNA copies close to the *LEU2* insertion result in a detectable event. The average displacement during unequal crossing over is seven repeats out of a total cluster length of 140 repeat units, so that only 7/140 of all events are seen. The estimated frequency of deletion is 5×10^{-4} per generation; therefore, the approximate frequency of unequal crossing over within the whole rDNA cluster is 10^{-2} per generation.

Two independent calculations of the rate of rDNA rectification, based on the measured frequency and extent of unequal crossing over, are described below. We have set the initial conditions for these calculations at a 1:1 ratio of two neutral alleles. The effect of successive deletions and duplications is to cause this ratio to drift, so that the probability distribution of the allelic ratio becomes broadened. Eventually, cell lineages accumulate in which one allele or the other has become fixed in the cluster. For the purpose of these calculations, all deletions and duplications were assumed to be of seven repeat units, and to occur in alternation and randomly throughout the cluster.

Ohta³⁵ has presented a simple method for calculating the mean time required for the fixation of a neutral allele in a cluster by the reiterated action of unequal crossing over. In this model the alleles are assumed to be randomly interspersed. The number of cross-over cycles required for fixation is given by

$$t = -\frac{1}{p} [n^2 (1-p) \log_e (1-p)]$$

where p is the initial frequency of the allele in question, n is the number of cross-over units in the cluster, and one cross-over cycle is defined as one deletion event and one duplication event. We let $p = 0.5$ for an initial ratio of 1:1, and $n = 20$, for a cluster of 140 repeats with cross-over of seven repeats. The time required is 280 cross-over cycles. One cycle requires two unequal cross-overs, which occur at frequency of 10^{-2} per generation. Thus the mean time required for cross-over fixation is 5.6×10^4 generations.

We have developed a method which allows us to calculate the probability distribution of the allelic ratio in a population as a function of time (A. Blodgett and J. W. S., unpublished results). We consider the rDNA cluster to consist of 20 cross-over units, with unequal cross-overs involving deletion or duplication of one cluster of seven repeated units (on average) at a time. The probability of every allelic ratio from 20:0 to 0:20 is specified in a linear array. This array is a vector $\mathbf{p}(n)$, describing the probabilities after the n th cross-over cycle. Initially the probability of a 10:10 ratio is 1.0, and all other ratios have a probability of 0. We then calculate a square transition matrix, π , containing the probabilities of change from any one allelic ratio to any other ratio after one cross-over cycle, such that $\mathbf{p}(n+1) = \pi \times \mathbf{p}(n)$, and $\mathbf{p}(n+k) = \pi^k \times \mathbf{p}(n)$. Thus by repeated squaring of the transition matrix, an operation simple to perform on a computer, we can calculate the probability distribution vector after 1, 2, 4, 8, 16... cross-over cycles. From this probability distribution, we can calculate the probability of drift past any particular allelic ratio. The results of these calculations are summarised in Fig. 5. Drift in the allelic ratio to fixation reaches a 50% probability at 240 cross-over cycles, or about 48,000 generations. This is in good agreement with the calculation by Ohta's method. The difference is probably because complete clustering (linkage) of like alleles was assumed in this particular calculation. Drift from the original 1:1 allelic ratio to a 2:1 ratio

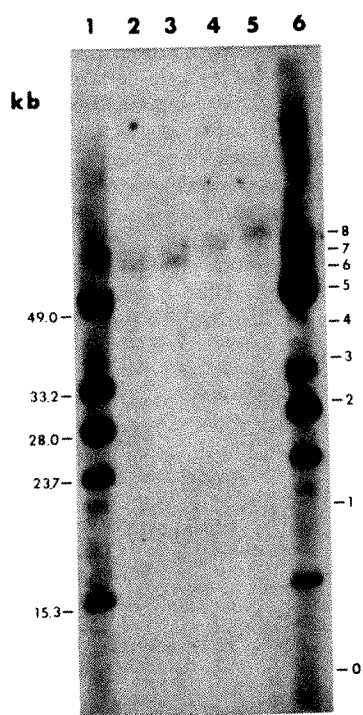


Fig. 4 Duplications produced by spontaneous unequal crossing over. The methods are as described in the legend to Fig. 3, with molecular weight markers in lanes 1 and 6. The strains used in this experiment all generate a discrete *Pst*I fragment that contains pBR322 sequences and therefore have a duplication of the insertion. The spontaneous duplication strains S21 and S14 (lanes 4 and 5) have duplications of 7 and 8 rDNA repeat units, respectively, calculated from the size of the *Pst*I fragment. Two subclones of strain S20 (lanes 2 and 3) have a duplication of 6 rDNA repeat units.

has a 50% probability at 5×10^3 generations, and a probability of 0.1 at 1,000 generations.

Discussion

We have recently shown that it is possible to integrate fragments of foreign DNA into the ribosomal RNA genes of yeast by transformation²⁵. Here we have described the use of these insertions for the analysis of unequal crossing over within the rDNA locus. Both rectification and magnification in repeated genes have been explained by unequal sister chromatid exchanges. The effect of a reciprocal unequal exchange is the generation of a deletion in one chromatid and a duplication in the other (Fig. 2). This process can lead to the elimination or fixation of a mutant allele in a cluster of repeats in the absence of selection. The process of cross-over fixation is analogous to the fixation of a neutral allele in a population by genetic drift, and is subject to a similar mathematical treatment. The variables affecting the rate of cross-over fixation by sister chromatid exchanges in a haploid are the frequency of unequal crossing over, the extent of mis-pairing (that is, the size of the deletions and duplications that are generated) and the number of repeat units in the cluster. The time required for the fixation of a neutral allele is proportional to the square of the number of cross-over units in the cluster.

The generation of deletions and duplications by unequal crossing over can also affect the copy number of repeated genes. The behaviour of the *bobbed* locus of *Drosophila*³⁶ has been studied extensively in this respect. This locus corresponds to the nucleolus organiser, the site of the tandemly repeated rDNA genes, and the *bobbed* mutation is due to a deficiency of rDNA. These deletions are unstable and revert to wild type by recombination. Tartof²⁴ has presented evidence that unequal crossing over is responsible for rDNA magnification in *Drosophila*: the

recombinational events occur in mitotic cells; gene reduction (decrease in copy number) occurs as well as copy number increase; and these events do not occur in a ring-X chromosome where the frequency of viable sister chromatid exchanges would be greatly reduced. The existence of a mechanism that is continuously generating small deletions and duplications at the rDNA locus of yeast suggests that there may be considerable variation in the rDNA copy number within a population. The average copy number in a culture is about 140 repeats per haploid genome³⁷. Natural selection alone may be sufficient to maintain the copy number near this average; alternatively some regulatory mechanism could keep the copy number closer to the optimum value.

The unequal crossing-over model of rectification and magnification does not require any new molecular mechanisms. Although sister chromatid exchanges are normally genetically invisible, they were first seen cytologically by Taylor³⁸ in his classical labelling experiments with *Vicia faba* chromosomes. Recently it has been suggested that chromosome labelling reagents such as ³H or bromodeoxyuridine cause DNA damage and induce the observed sister chromatid exchanges (SCEs). However, Yamamoto and Miklos³⁹ have detected SCEs in *Drosophila* in a ring-Y chromosome. Dicentric chromosomes resulting from sister exchanges were observed at a frequency of 0.3% in the absence of any agents which might be expected to induce this process. Unequal crossing over is familiar in *Drosophila* where it was first observed during genetic analysis of the *bar* duplication. The occurrence of unequal sister chromatid exchanges in a repeated gene cluster is therefore not surprising; in contrast, all other models of rectification require new and more complex forms of recombination.

We have measured the size of the duplications generated by unequal crossing over in yeast rDNA. In all of the events that were analysed the two copies of the insertion were relatively close to each other. The non-random distribution of the two copies has two implications. First, duplications caused by looping out of an rDNA circle including the insertion, followed by its reintegration in the sister chromatid, can be only a small fraction of the events. This mechanism would result in the two copies of the insertion being randomly distributed with respect to each other in the rDNA cluster. Second, the rDNA region of the two chromatids must pair in an ordered manner before

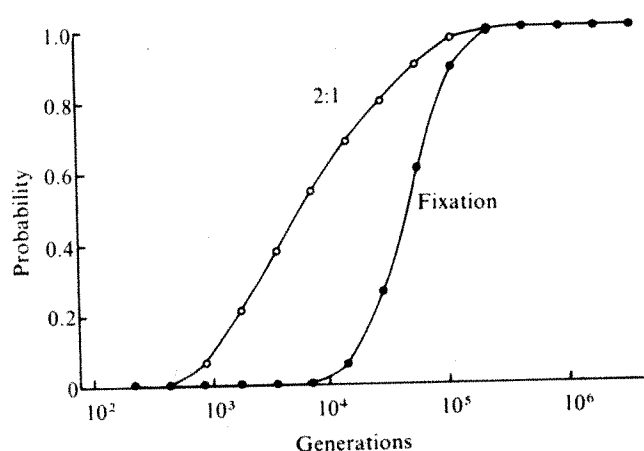


Fig. 5 Computer projections of the rectification process in yeast rDNA. The probability of drift from an initial 1:1 ratio of two alleles to a 2:1 ratio or to complete fixation was calculated as a function of time. These calculations were based on measurements of the rate and extent of unequal crossing over as described in the text. We have assumed two neutral alleles, with like alleles linked (clustered), and that deletions and duplications occur alternately, randomly throughout the cluster, and without effect on the growth rate of the cell. Drift in the allelic ratio then occurs by the random action of repeated small deletions and duplications. Drift to fixation reaches a probability of 0.5 by 4.8×10^4 generations. Drift to a 2:1 ratio should be detectable at 10^4 generations, but is unlikely to be seen at 10^3 generations.

recombination. If any rDNA repeat could pair equally well with any other, unequal crossing over would generate very large deletions and duplications that would probably be deleterious to the cell.

The frequency and extent of unequal crossing over are the two parameters required to describe the process of rectification during mitotic growth of haploid yeast. The calculations presented above and in Fig. 5 show that starting from a 1:1 ratio of two alleles in a cluster, the probability of fixation for either allele is 0.5 at 3.7×10^4 generations, and the probability of drift to a 2:1 ratio in either direction is 0.5 at 4.3×10^3 generations. The probability of drift to a 2:1 ratio after 1,000 generations is 0.1. This projection is consistent with the result of the following

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experiment. Petes and Botstein⁴¹ have identified a diploid strain of yeast that is heterozygous for a sequence change in the rDNA, detected as a restriction enzyme site variant. They were able to find recombinant spores heterogeneous for their rDNA. Petes (personal communication) cultured subclones of a haploid strain with a 1:1 ratio of two alleles for 1,000 generations. No change in the allelic ratio was observed. Therefore the actual frequency of unequal crossing over cannot greatly exceed the frequency measured with the rDNA insertion.

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LETTERS

Evolution of the inclination of Phobos

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Two mutually exclusive hypotheses concerning the origin of the martian satellites have commonly been proposed: (1) they were formed in the vicinity of Mars, on orbits not very different from their present orbits, or (2) they originated far from Mars and were somehow captured^{1–3}. Hypothesis (1) had been favoured because the satellites' orbits are nearly circular and equatorial. More recent Viking results imply that the satellites are composed of carbonaceous material^{4,5}, suggesting an asteroidal origin. We show here that the satellites have remained close to their laplacian plane throughout their orbital evolution. Thus Phobos and Deimos need not have originated near the martian equator as previously believed³, but could have been captured in Mars' orbital plane instead, and later evolved to their present inclinations. This new result seems to make hypothesis (2) more plausible.

A substantial secular acceleration of Phobos has been observed^{6,7}, presumably caused by tidal dissipation in the interior of Mars. However, this mechanism seems unable to account for a drastic orbital evolution over the age of the Solar System, particularly for Deimos^{3,8}. On the other hand, Lambeck³ has shown that tides raised by Mars in the satellites, especially in Phobos, can lead to substantial changes in both the semi-major

axis and eccentricity. Because of the tidal coupling between these two elements, Phobos may once have had a very large eccentricity (≥ 0.5), permitting its semi-major axis to decrease across the synchronous orbit. Apparently Deimos has always been outside the synchronous orbit for Mars, but in the far distant past both its eccentricity and semi-major axis may also have been great. Lambeck³ concluded that Phobos, and possibly Deimos, may have been captured into an initial nearly parabolic orbit which subsequently evolved to the present orbit. However, as previously pointed out^{3,8}, tidal interactions alone cannot change the satellites' inclinations to the martian equator by more than a few degrees over the age of the Solar System. This leads to a serious objection to the capture hypothesis: if Phobos and Deimos have been affected only by tides, their initial capture orbits must have had nearly the same orientation as the martian equator, which seems improbable.

In fact the apparent small changes in the satellites' inclinations result from neglecting the precession of their orbits and the obliquity of Mars. Laplace^{9,10} has shown that a satellite orbit maintains a nearly constant inclination to its proper (laplacian) plane during a nodal precession period. This plane lies between the equatorial and orbital planes of the primary and shares a common node with them. The inclination I of the laplacian plane to the planet's equator is given by⁷:

$$mJ_2R^2a^{-3} \sin 2I = \frac{1}{2}Ma^2a_M^{-3} \sin (2\theta - 2I) \quad (1)$$

where m is the mass of Mars, J_2 its coefficient of dynamical oblateness, and R its radius; a is the semi-major axis of the satellite's orbit; M is the mass of the sun; a_M is the semi-major axis of the planet's orbit; θ is the planet's obliquity, the angle between its equatorial and orbital planes. Terms of second order in the planet's eccentricity, and of fourth order in the satellite's have been neglected in equation (1).

As the orbits of Phobos and Deimos are evolved backwards, the influence of the Sun on their motions increases while the perturbations due to the oblateness of Mars decrease. At a critical distance of about 13 martian radii¹¹, the laplacian plane switches from near the martian equator to nearer Mars' orbital plane. Because the tidal interaction does not strongly affect the inclinations of the orbits with respect to the laplacian plane, the satellites approach the martian ecliptic as they evolve back beyond the critical distance. When the satellite's eccentricity is included in the definition of the laplacian plane, the critical distance apparently increases to ~ 14 martian radii.

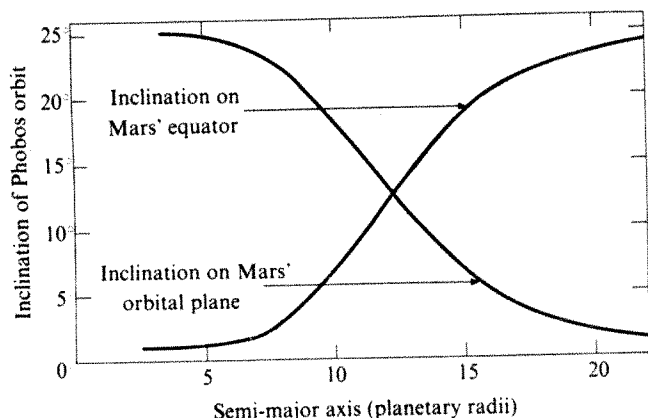


Fig. 1 Variation of the inclination of Phobos' orbit on the equatorial and orbital planes of Mars, versus its semi-major axis. For simplicity, the satellite's eccentricity has been neglected in calculating the laplacian plane.

To study the evolution of the martian satellites backwards in time, we have numerically integrated their semi-major axes, orbital eccentricities, and inclinations with respect to the laplacian plane, and simultaneously expressed their inclinations with respect to the martian equator and orbital plane. We considered both tides in the planet and tides in the satellites, using the tidal expansion of Mignard¹². This method has the capability of expressing the tidal potential relative to any reference frame; in this case, it is applied to the laplacian plane. Because Phobos and Deimos are in synchronous rotation, we have assumed that their spin axes coincide with their orbital poles throughout the orbital evolution. Thus neither tides in the satellites nor precession can alter their inclinations to the laplacian plane. The detailed method of calculation will be presented elsewhere.

The history of the semi-major axis and eccentricity depends on the frequency dependence of the tidal model. Our results are partly similar to those of Lambeck³, indicating that the eccentricity of the martian satellites may have been very high in the past. However, the orbital inclinations on the laplacian plane always remain small. Figure 1 shows the resulting variation in the inclination of Phobos' orbit to the equator and orbit planes of Mars, for a representative calculation.

As the orientation of the laplacian plane depends on the distance from Mars, we see that the orbital inclination to the equator increases beyond the critical point up to about 25°, the average obliquity of Mars¹³. At the same time, the inclination to the orbital plane of Mars falls to $\sim 1^\circ$. A similar result is expected for Deimos, but the time scale of evolution is far longer. This raises the possibility that the orbits of Phobos and Deimos crossed in the past^{3,8}.

Thus our results indicate that the martian satellites originated very close to the martian orbital plane if they ever have been outside the critical distance of Mars. In particular, this conclusion supports the capture hypothesis.

While we have replaced one 'special' plane (the martian equator) with another (the martian orbit), it is plausible that a

conceivable mechanism for capture would favour the ecliptic. In comparison, there is no obvious reason why capture should occur preferentially into the martian equator. On the other hand, the accretion hypothesis supposes that the primordial satellites' orbits lay in the laplacian plane, which depending on the distance from Mars, approaches either the equatorial or orbital plane. However, in the latter case, accretion would have occurred beyond the critical radius, which seems unlikely according to current accretionary models. Thus, while not inconsistent with an *in situ* formation, our results have removed a major difficulty with the hypothesis that Phobos and Deimos are captured asteroids.

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Radiogenic melting of primordial comet interiors

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Comets accreted soon after the initial collapse and cooling of the solar nebula, and containing a plausible fraction of ^{26}Al , would have been significantly heated as this radionuclide decayed. Snow-and-dust balls as described by integrals of the heat conduction equation would melt in the centre if larger than 3–6 km radius. A central, low pressure vapour-droplet mixture is described here, which is conceived to be retained within an ice shell, and providing a potentially hospitable environment for elementary life forms. Refreezing after some million years produces a partially-hollow core.

Cooling and condensation of the solar nebula is thought to have occurred very rapidly, <1 Myr after nucleosynthesis of a component of the Solar System material¹. On Whipple's icy-conglomerate theory (for a recent review see ref. 2), comets also condensed at this early stage out of frozen gases mixed with solid material similar to carbonaceous chondrites, including primordial radioactive components. Whipple and Stefanik³ did investigate the probable heating due to radioactive decay, mainly by ^{40}K for they assumed a 20 Myr interval before condensation, so deriving maximum central temperatures of a few tens of Kelvins for 10-km radius icy comets. Sub-surface cometary material would thus have retained its initial pristine state—the examination of such unevolved material being a principal goal of planned cometary missions.

However, present evidence implies significant amounts of the much more effective radionuclide ^{26}Al were present in early Solar System condensates⁴, quite possibly in sufficient abundance to melt the interiors of comets⁵. This element is rather short-lived (half-life 7.4×10^5 yr) and delivered some $2,000 \text{ cal g}^{-1}$ to chondritic meteorites⁴ (assuming all the β^+ and γ -ray energy to go to heat the radio-isotope fraction to be 6×10^{-5} and the relative Al abundance of ref. 6). Comets, if accreting homogeneously out of this material⁶ with a sizeable

proportion of ices (see legend to Fig. 1), would have been subject to a smaller uniform heating rate

$$Se^{-\lambda t} \text{ where } S/\lambda = 2,000\eta \text{ cal g}^{-1} \\ \lambda^{-1} = 3.2 \times 10^{14} \text{ s}, \eta = 0.1-0.5 \quad (1)$$

This rate is $\sim 10^4$ times higher than that from ^{40}K and so on considered earlier³. To bring ice from low temperatures to melting point 90 cal g^{-1} is required and a further 80 cal g^{-1} is necessary to melt it. Thus the heat S/λ may be sufficient to melt the centre of an initial snowball comet if thermal conductivity is low and the mineral fraction is substantial, $\eta > 0.1$.

The thermal conductivity of an ice-dust comet is poorly determined because of its constitution and compactness. To secure analytical solutions I assume as previously^{3,7,8} representative constants for the density and conductivity:

solid ice + dust ($\sim 200 \text{ K}$):

$$k = 7 \times 10^{-3} \text{ cal cm}^{-1} \text{ s}^{-1} \text{ K}^{-1}, \rho = 1.0 \text{ g cm}^{-3}$$

loose snow:

$$k = 4 \times 10^{-4} \text{ cal cm}^{-1} \text{ s}^{-1} \text{ K}^{-1}, \rho = 0.25 \text{ g cm}^{-3} \quad (2)$$

with specific heat $c = 0.35 \text{ cal}^{-1} \text{ g}^{-1} \text{ K}^{-1}$, appropriate to 200 K ice. While for ice c varies strongly with temperature, for the present purposes it is only significant that k for ice is much higher than for snow.

Consider first a spherical comet of loose snow and mineral grains. The temperature in the case of uniform k is given adequately by the particular integral of the diffusion equation⁹:

$$T = T_0 + \frac{S}{c\lambda} (a \sin ar / r \sin aa - 1) e^{-\lambda t}, \alpha = (\rho c \lambda / k)^{1/2} \quad (3a)$$

$$= T_0 + \frac{S\rho}{6k} \left\{ a^2 - r^2 + a^2 O(\alpha^2 a^2) \right\} e^{-\lambda t} \quad (3b)$$

T_0 is the surface temperature, somewhat higher than the local radiation temperature T_r , which itself is just a few Kelvins far from the Sun in the 'Oort cloud' of comets, but could be some tens of Kelvins if the comets condensed early in the cooling phase of the supernova and solar nebula gases¹. The maximum, steady state value of T_0 given by radiation balance is

$$T_{0,\max} = \{T_r^4 + \rho a S / 3\sigma(1-A)\}^{1/4} \quad (4)$$

which for 10-km snowball with $\eta = 0.4$, albedo $A = 0.2$ and negligible T_r gives $T_{0,\max} = 37 \text{ K}$. Transient terms, dependent on unknown initial conditions, have been omitted from equation (3), as this is applicable for times greater than $\rho c a^2 / \pi^2 k = 7 \times 10^3 \text{ yr} \times (a/1 \text{ km})^2$ and for the source decaying little over a diffusion time $[\alpha a \leq \pi/2]$. The scale distance is $\alpha^{-1} = 4 \text{ km}$, so taking the lowest terms in the expansion (3b) is not too accurate for kilometric comets. The centre of this loose snowball comet could melt were the temperature raised by $\Delta T = 273 \text{ K} - 37 \text{ K} (\eta a / 4 \text{ km})^{1/4}$ that is if

$$\frac{\alpha a}{\sin aa} > 1 + \frac{c\Delta T}{S/\lambda}, \text{ implying } a > 3-6 \text{ km} \quad (5)$$

comparable with that suggested previously⁵.

Could the pressure be high enough— $>6 \text{ mbar}$ of the triple point—to allow liquid H_2O ? The hydrostatic pressure due to self-gravity is marginally adequate: $\frac{2}{3}\pi\rho^2 G(a^2 - r^2) = 9(1 - r^2/a^2) \text{ mbar}$. A degree of cohesion between the snow crystals is also to be expected. For vapour diffusing outwards would recondense in the cooler outer layers. As cracks and interstices fill up with condensates, the interior pressure would rise, fissures may develop allowing some outgassing, but further condensation would reseal them. Thus, initially loose snow would be compacted and consolidated into an icy layer possess-

ing tensile strength, readily able to maintain pressures higher than the 6 mbar .

The relatively highly conductive (and perhaps convective) fluid and ice interior and loose snow exterior is most simply described by a two layer model: the solution corresponding to equation (3) for a shell of radius a surrounding a core of radius b , conductivities k_a, k_b is

$$T = \frac{S\rho}{6k_a} e^{-\lambda t} \frac{a^2 - b^2 + (b^2 - r^2)k_a/k_b}{a^2 - r^2} \quad \begin{matrix} r < b \\ b < r < a \end{matrix} \quad (6)$$

with $T(b) = 273 \text{ K}$ at radius b . For $k_a/k_b \ll 1$ (for example, $1/70$ by equation (2)), the interior temperature is nearly constant (273 K) and the shell temperature is a little less than the previous expression (3b). The latent heat L required in the melting or the sublimation of the ice also modifies the internal temperature: a term expressing the fact that the source has decayed while the latent heat is being delivered can be derived⁹. This term is smaller by the factor $6\lambda L b^2 / S a^2 = 0.25(1 - \eta)b^2 / \eta a^2$ than the main term, and expresses a reduced radius b for the liquid core.

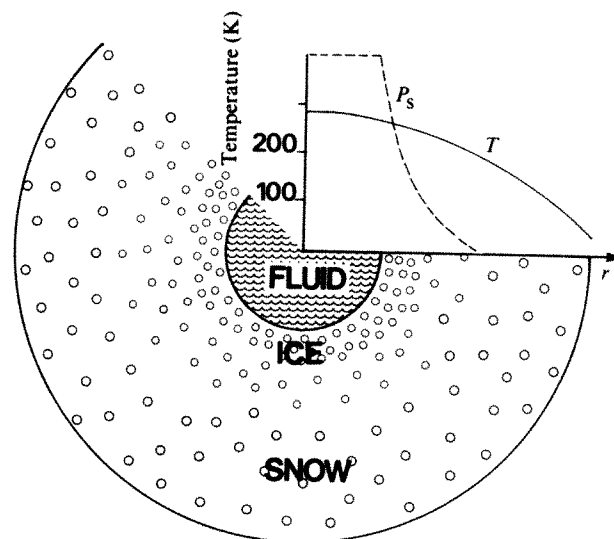


Fig. 1 A snowball comet of around 10-km radius (10^{18} g) during the initial melting phase. The temperature T and vapour pressure $p_s \sim \exp(-2,720 \text{ K}/T)$ (ref. 7) are calculated assuming convection is effective in the central vapour-droplet fluid. Comets are presumed to have accreted at a similar period to chondritic meteorites but have lower ^{26}Al radiodecay heating by a factor $\eta = 0.5-0.1$. For along with the mineral content, they retained volatiles in the form of ices of rather greater or comparable mass². The estimate for η encompasses the value $0.4-0.45$ derived if sufficient O and C to make up solar relative abundances are added to the constituents of carbonaceous chondrites⁶. The parametric values (2) are used in calculating the outer snow layer¹⁵.

The structure of a melting nucleus is certainly more complex than this two-layer model. For one thing, water is much denser (factor 4) than the snow. The gravitational pressure of a few mbar is surely too low to crack open the ice shell. Ice is a good conductor see equation (2) and in melting, readily absorbs heat from the interior fluid so the H_2O vapour pressure is probably not much higher. Could there be a central core of water surrounded by vapour? Surely not, for the gravitational potential in the vapour is flat, so the core would splash against the ice sides and fragment into drops. The central region must therefore be a mixture of one part dust and droplets, three parts vapour. The energies in droplets' dynamic motion and surface tension is of order of the potential difference: for example, typical speeds are $\{\frac{2}{3}\pi\rho Gh^2\}^{1/2} \sim 0.2 \text{ cm s}^{-1}$ if the size h of convective cells is guessed as 10 m . The ice to snow transition is also complex. Under the vapour pressure gradient (Fig. 1) sublimation with diffusion outwards and condensation in cooler outer regions is expected,

just as in snow under sub-zero air temperatures on Earth¹⁰. This means a metamorphosis of the snow into larger ice crystals and enhanced thermal conductivity due to vapour transport:

$$k_{\text{eff}} = k + \zeta \frac{L}{RT} \frac{\partial p_s}{\partial T}$$

Assuming $\zeta = \varepsilon v_{\text{th}}/3$, with $\varepsilon = 1$ mm a typical hole size, the additional conductivity is found to exceed k for snow, equation (2), above 200 K. Thus in the 10-km snowball sketched in Fig. 1, a 1-km thick icy layer with enhanced conductivity is expected, and a further 2 km of metamorphosing snow.

There may be additional chemical heating which counteracts latent heat effects. If there had been little chance for radiative processing and polymerisation of the icy grains¹¹ then states of lower chemical energy would be accessible in the vapour-liquid-mineral dust interior, through catalysed chemical and perhaps biological^{8,12} processes. Supposing Solar System abundance of C and 0.2–0.5 eV per C-atom (compare with Van der Waals forces at 0.2 eV per bond; H₂CO polymerisation at 0.37 eV) the extra heat amounts to 80–200 cal^{−1}. Being released quickly from newly-melted material, it makes the effective latent heat negative and may fully counterbalance the thermal capacity (90 cal g^{−1}). If so, once melting has started, the liquid-vapour core grows very quickly (the chemical time scale) and equation (6) describing a fully-developed core is rapidly obtained ($\lambda t \ll 1$).

As the radioactivity decays, the comet centre gradually refreezes, with an exponential decay time of λ^{-1} . Convection may enhance thermal transport, but anyway the ice-water-vapour interior is relatively highly conducting, so the shell-core solution (6) with $k_a \ll k_b$ is appropriate. Allowance for the latent heat can again be made⁹, but only expresses a slowing down in refreezing. In structural terms, freezing proceeds through deposition of frost and hail on the interior of the ice shell, resulting eventually in a hollow core.

Thus a 10-km snowball comet (10⁶ g) would have developed a large fluid core with a 1-km thick icy shell, while the snow surrounding it underwent extensive processing through sublimation, diffusion and recondensation, before the core refroze (with crystallisation effecting further differentiation) in ~ 1 Myr. The hollow core resulting on refreezing provides an explanation for the splitting of some disintegrating comets into fragments of comparable size^{3,13}. Of those observed over the past 25 yr, comets Wirtanen, Humason, Schwassmann-Wachmann 1¹⁴ and Chiron (if it is a comet) seem big enough to have possessed large fluid cores, although comets such as Encke and Halley may have been bigger before centuries of outgassing. The central fluid core would have provided an excellently-protected environment for elementary biological systems¹², particularly for colonies of bacteria which could survive the refreezing and 4,500 Myr isolation, and be released from comets evaporating and disintegrating on approach to the Sun.

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A search for ultra high-energy γ -ray bursts from celestial sources

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We report here the details of an experiment carried out at mountain altitude, at Ootacamund (2.2 km above sea level), India between March 1977 and March 1979, to detect bursts of high energy ($\sim 5 \times 10^{13}$ eV) γ rays from celestial sources. Not having detected any such bursts, we give 99% confidence level upper limits both to their flux and to the primordial mini-black hole explosion rate in the vicinity of the Solar System.

The apparatus consists of four 20-cm deep liquid scintillation detectors, each 1 m² in area located at the corners of a square of side 11 m. Each scintillator is viewed by a RCA 8575 photomultiplier with the associated electronics set at a threshold of 0.33 of the average pulse height for a vertically penetrating incident cosmic ray muon. The scintillators are, therefore, fully sensitive to single charged particles. Low-energy air showers are selected by a fourfold coincidence of the pulses from the scintillators with a resolving time of 1 μ s. The observed shower rate is 0.55 s^{−1} with a negligible contribution from chance coincidences. The primary energy threshold is difficult to calculate precisely; although we can approximate it at $\sim 5 \times 10^{13}$ eV. We looked for events in which a rapid sequence of air showers occurred with a short time separating them. Specifically all events in which three or more showers occurred with inter-shower time separations of 10 ms or less were selected by the burst detection system discussed in detail elsewhere¹. The event times were recorded to an accuracy of 1 ms in UTC (coordinated universal time) and the inter-shower time separations to an accuracy of 1 μ s. The expected chance event rates of the '3', '4' and '5 or more' type bursts due to just poissonian fluctuations are 1.44, 7.9×10^{-3} and 4.3×10^{-5} per day respectively. Thus while the '3' and '4' type of events are trivial, a '5 or more' type of burst is very significant if recorded in an operation period of a year or two. To discriminate against spurious bursts generated by local phenomena like electrical discharges, a dummy system with electronics identical to that of the burst detection system (except that the photomultipliers were absent) was also operated during the experiment. A signal from any one of the four dummy detectors was used to flag out a possible noise event.

In an effective operation time of 4.78×10^7 s (1.51 yr), no '5 or more' type of burst was observed. The numbers of '3' and '4' type of events observed during the same period are 810 and 4 compared with the expected 797 and 4 events respectively due to chance. The absence of any '5 or more' type of bursts allows us to set an upper limit on the frequency of γ -ray bursts.

The 99% confidence level upper limit to the flux of γ -ray bursts, characterised by at least five γ -ray photons each of energy $\geq 5 \times 10^{13}$ eV arriving within a time interval of 0.1 s, is

$$\frac{4.6}{\pi(30 \text{ m})^2 \times 4.78 \times 10^7 \times 1} = 3.4 \times 10^{-11} \text{ m}^{-2} \text{ s}^{-1} \text{ sr}^{-1}$$

Here we assumed that the collection area is that of a circle with a radius of 30 m and the solid angle, 1 sr.

It is obvious that the same results can be used to set an upper limit to the number of exploding mini-black holes postulated by Hawking^{2–8}. Using the same calculation procedure as Fegan *et al.*⁹, who carried out a similar experiment, we arrive at a 99% confidence limit of 2.7×10^3 mini-black hole explosions pc^{−3} yr^{−1}. This compares favourably with the limit of 6×10^3 pc^{−3} yr^{−1} set by Fegan *et al.* for a threshold energy of

$\sim 10^{14}$ eV. Porter and Weekes¹⁰, using an atmospheric Cerenkov technique, obtained an upper limit for mini-black hole explosion rate of $3 \times 10^4 \text{ pc}^{-3} \text{ yr}^{-1}$ at a threshold of 5×10^{12} eV. According to Page and Hawking⁷ and Carr⁸ who discussed in detail the observable consequences of primordial mini-black holes in the so-called elementary particle model, 10–30% of the rest mass (10^{15} g) of an exploding black-hole will result in the emission of a burst of about 10^{30} photons each of energy $\sim 5 \times 10^{12}$ eV, over a time scale of 0.1 s. The above limits have to be considered in the context of this prediction.

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Electrogenerative reduction of nitric oxide

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Electrogenerative processes are those in which favourable thermodynamics and kinetic factors are utilised in an electrochemical cell to generate by-product electricity while bringing about a desired reaction. The cell actually functions as a reactor to produce a desired chemical or occasionally to destroy an undesired one. Electrode potentials are generated by reactions at the electrodes; they are frequently different from those of conventional electrosynthesis. Here, we describe an exploratory study of the electrogenerative reduction of nitric oxide to favour ammonia production as well as the effect of electrocatalyst, external load, and other parameters on product distributions.

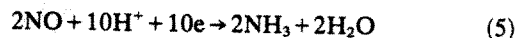
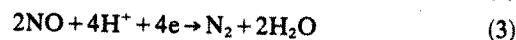
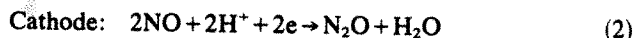
Nitric oxide can be produced in several ways. One method would be through concentration from a polluting effluent gas stream. Another possibility would be passage of an effluent stream through an electrogenerative cell removing nitric oxide while generating d.c. electricity.

The reduction of nitric oxide has been of special interest from a basic and environmental viewpoint¹, both electrochemical reductions^{2–5} and conventional catalytic hydrogenations^{6–10} having been investigated. For the 'electrogenerative' reduction of nitric oxide, the free electrolyte phase is bounded by porous Teflon-backed, gas-permeable, liquid-impermeable, electrodes which are connected through an external variable resistor in series with an ammeter¹¹. Hydrogen at atmospheric pressure reacts in one electrode compartment, while nitric oxide reacts at the other with protons and electrons from the external circuit to generate current at potentials determined by reactions at each electrode. Details of cell construction for constant gas flow at atmospheric pressure are given elsewhere¹².

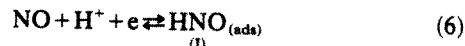
Identified overall electrode reactions can be represented by



H^+ transported through the electrolyte



By varying external cell resistance and monitoring the current (conversion rate) of the external circuit, polarisation curves can be obtained as shown in Fig. 1 for cells with platinum and ruthenium cathodes and perchloric acid electrolyte. Gas was fed continuously at both electrodes. The open circuit potential of ~ 0.93 V is reminiscent of that for a hydrogen-oxygen fuel cell and may reflect dissociative adsorption at the cathode to give N_{ads} and surface oxygen^{4,13,14} or possibly is due to the intermediate reaction



HNO intermediates have been postulated to decompose to give nitrous oxide and to be reduced by an alternate route to ammonia⁶ or hydroxylamine^{2–5}.

With some polarisation runs, product analyses were obtained for constant current over several hours. Gas chromatographic (GC) analyses for NO, N_2O and N_2 were done on cathode compartment (volume = 0.78 cm^3) outlet and inlet streams on Porapak Q columns. Ammonia and minor amounts of hydroxylamine accumulating in the electrolyte were measured using standard colorimetric methods^{15,16}. Most material balances on products accounted for generated current within 10% or better.

Some results for platinum and ruthenium cathodes (3.88 cm^2 apparent area) at constant current are shown in Table 1. The platinum black electrode was the commercial, American Cyanamid, Teflon-backed LAA-2 type^{17,18} (9 mg cm^{-2}). The ruthenium black electrode (9 mg cm^{-2}) was an AA-2 (refs 19, 20) type mechanically backed with porous Teflon. Possibilities of employing the electrogenerative cell as a chemical reactor are illustrated by Run 1 with platinum where the majority of the feed is converted to nitrous oxide with little ammonia formation. The effect of nitric oxide flow rate variation at lower potential is illustrated by Runs 3 and 4 on platinum where either ammonia or N_2O can predominate. This can be rationalised by strongly adsorbed nitric oxide^{7–9} displacement of surface intermediates with faster flow rates to give less reduced species and longer surface contact time at slower flows to produce more reduced species. The lower potential further favours ammonia formation. In Run 5, sulphuric acid electrolyte with platinum did not give results significantly different from perchloric acid. Results for NO reduction on ruthenium illustrate electrocatalytic specificity towards ammonia formation. Its unusual adsorptive properties for nitrogen and reduced intermediate species^{7,8,21} diminish the dependence of ammonia formation on flow rate.

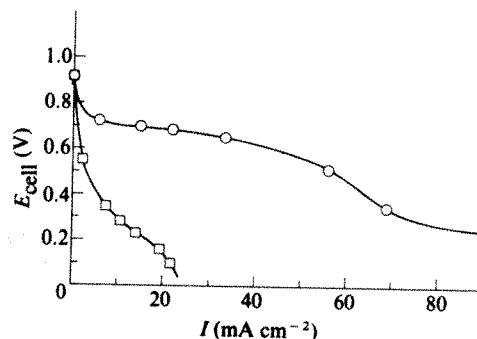


Fig. 1 Current density-voltage curve for electrogenerative reduction of nitric oxide at 25 °C. IR corrected, platinum black anode, 2M HClO_4 aqueous electrolyte, 0.6 cm thick, $R_{\text{int}} = 0.39 \Omega$; \circ , Pt black; \square , Ru black cathode. NO feed $4.6 \text{ cm}^3 \text{ min}^{-1}$. (See text for details.)

Table 1 Nitric oxide-hydrogen electrogenerative cell operational data

Expt	Cathode**	Electro-lyte*	NO (cathode) feed rate, (cm ³ min ⁻¹)	Cathode potential† (V)	Current density (mA cm ⁻²)	Fraction of total current‡ (%)				Calculated§ coulombic efficiency	NO conversion (%)
						N ₂ O	N ₂	NH ₂ OH	NH ₃		
1	Pt	C	3.6	0.56	52	98.3	0.0	0.0	1.7	96.2	83
2	Pt	C	1.6	0.50	26	78.4	20.3	0.0	1.3	94.4	97
3	Pt	C	3.8	0.21	84	57.8	20.8	1.1	20.4	98.4	93
4	Pt	C	1.5	0.22	63	18.4	24.7	1.8	55.2	95.2	99
5	Pt	S	1.8	0.27	80	14.9	21.6	1.8	61.7	88.0	96
6	Ru	C	3.8	0.23	12	21.1	4.8	0.0	74.1	100.5	10
7	Ru	C	1.6	0.23	14	21.1	0.4	3.8	74.8	93.5	23
8	Ru	C	1.6	0.10	28	7.3	3.7	2.6	86.5	102.2	34
9	Pt	S	0.08	0.12	24¶	0	0	0	13.5	100.3	>99.5*

* C = 2M/HClO₄, S = 3M/H₂SO₄.

† Relative to hydrogen electrode.

‡ Normalised to 100%.

§ Based on generated current; analysis of cathode gas streams and electrolyte.

|| Overall feed rate is 4.5 cm³ min⁻¹, mixture contains 1.9% NO, 8.6% O₂ in N₂.¶ Involves substantial O₂ reduction.

* Final NO concentration in product gas stream was less than 100 p.p.m. (limit of GC detectability) after single pass through cell.

** Pt black anode, see text.

Ammonia formation favoured by ruthenium makes electrogenerative reactor use attractive in conjunction with thermal conversion of nitrogen and oxygen to NO (ref. 22) to produce ammonia.

Reductions here take place at generated potentials positive to the hydrogen electrode as compared with the frequent cathodic potentials of conventional electrochemical reductions. With reactants separated in contrast to heterogeneous catalytic reductions, reactant competition for adsorptive sites is minimised, allowing thermodynamic factors to operate across the interface to favour reaction. One consequence is the controlled reaction at mild, room temperature conditions. Possibilities for reacting other nitrogen oxides as well as nitrogen and hydrogen to form ammonia in the electrogenerative mode are suggested by these results. The nitrogen-hydrogen reaction in particular may be favoured through minimisation of adsorptive competition.

The high nitric oxide conversion with platinum cathodes raises the possibility of using electrogenerative reactors as a means for NO_x pollution control at stationary power plants. To pursue this, a cathode feed mix of 1.9% nitric oxide and 8.6% oxygen with nitrogen was used with the cell of previous runs in Run 9, where nitric oxide concentration was reduced to less than 100 p.p.m. (0.01%) in a single pass in the presence of oxygen. The high NO conversion involved NO oxidation to NO₂ in the feed stream as well as electrogenerative reduction of NO₂ and remaining NO to ammonia, combined with current generating oxygen reduction to water. Nevertheless, preferential NO reduction in the presence of oxygen should be noted. With the cell at open circuit, NO concentration was not reduced below 1,100 p.p.m. by O₂ oxidation even further downstream. The reduction in NO concentration with the electrogenerative cell is significant in view of present environmental concern over NO_x pollutants²³. Investigations of straight NO electrogenerative reductions as well as simulated pollutant mixtures are continuing.

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Leaching behaviour of rhyolite glass

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Glass has long been recognised as a potentially suitable matrix for diluting and stabilising nuclear waste¹⁻⁵. Most of the glasses which have been studied are borosilicates¹⁻³. One notable exception is the aluminosilicate nepheline syenite glass, used in the pioneering Canadian study⁴ that showed much lower apparent leach rates than for borosilicates^{4,5}. The natural aluminosilicate volcanic glass, rhyolite, has existed in some natural environments for millions of years^{6,7}; and, as Mendel has pointed out¹, it could be adopted as a model against which other glasses could be compared for nuclear waste disposal. We report here results on the hydrothermal leaching and recrystallisation of rhyolite at temperatures up to 300 °C, using not only the classical leach monitors of weight loss and solution analyses, but also modern surface probes such as SEM (scanning electron microscopy), ESCA (electron spectroscopy for chemical analyses) and SIMS (secondary ion mass spectroscopy). These surface techniques are especially valuable for gaining a more complete understanding of hydrothermal reactions of glasses or minerals⁸⁻¹⁰.

The quaternary rhyolite sample from Mono Craters, California, was found to be homogeneous with the following composition (wt %): 4.2% Na₂O; 12.4% Al₂O₃; 76.9% SiO₂; 4.6% K₂O; 0.6% CaO; 0.8% FeO (total 99.5%). Either 0.2 g, 0.5 g or 1.3 g disks were drilled, cut, polished and cleaned ultrasonically in an acetone bath. The disks were placed in stainless steel autoclaves along with either (1) ~10 ml of distilled water (pH ~5.8), or (2) 5 g of crushed granite (composition 71.3% SiO₂; 14.4% Al₂O₃; 2.68% Fe₂O₃; 1.43% MgO; 1.68% CaO, 3.43% Na₂O; 3.95% K₂O; 0.07% MnO; 0.11% P₂O₅; 0.41% TiO₂)¹¹ plus 10 ml of distilled water. The autoclave was sealed with a stainless steel cone, and placed in an electrically heated Nichrome V tube furnace at temperatures of 100, 200, or 300 °C for up to 1 week.

The pH of the solution was recorded after cooling; and the aqueous phase was diluted to 50 ml with ~1% HCl. This solution was analysed for Na and Si using atomic absorption, and the glass disks were examined using the Philips 501 SEM, and ESCA 36 spectrometer, and the Applied Research Laboratories ion microprobe mass analyser at the Metaal Instituut TNO, Apeldoorn, Holland.

Table 1 Weight loss (%) of rhyolite disks after leaching in H₂O and granite plus H₂O*

Test condition	Leach time	T (°C)	% Weight loss
H ₂ O	1 hour	100	≤0.01
	2 hours	100	≤0.01
	6 hours	100	≤0.01
	1 day	100	0.01
	2 days	100	0.01
	3 days	100	0.05
	1 week	100	0.08
	1 day	200	<0.1
	3 days	200	0.9
	1 week	200	0.9
	1 day	300	0.16
	3 days	300	3.5
	1 week	300	7.0
	1 week	100	<0.1
Granite plus H ₂ O	1 day	200	<0.1
	3 days	200	0.63
	1 week	200	0.37
	6 hours	300	2.6
	3 days	300	23.5
	1 week	300	31.8

* The 100 °C experiments in distilled H₂O were performed with disks 1.2 cm in diameter and 0.5-cm thick, having a surface area of 4.1 cm² and weighing ~1.3 g. All other leaching experiments were performed with disks 8 mm in diameter and 2 mm thick having a surface area of 1.5 cm² and weighing ~0.2 g.

The weight losses for the leached rhyolite are given in Table 1. At 100 °C, there was very little weight loss, and the rhyolite leach rate at 100 °C of 3.6×10^{-5} g cm⁻² d⁻¹ (Table 2) was ~3–10 times less than those for borosilicate disks studied earlier^{2,5}. At 200 and 300 °C, there was an appreciable weight loss, and noticeable crystalline material appeared after three days of leaching in experiment (1). In the water-plus-granite experiment there was no noticeable crystalline material formed even after 1 week. The pH of the leachate increased slowly at 100 °C from 5.8 to 7.8, and the Na leached into solution from the 0.5 g sample increased approximately linearly with time at 100 °C (for example 3.1, 6.5 and 35.2 µg Na after 6 h, 1 day and 1 week leaching respectively).

The leach rates based on Na at 100 °C are slightly higher than the weight-loss leach rates (Table 2), indicating some selective leaching of Na. However, at 200 °C, the Na and weight loss leach rates are very similar, indicating congruent dissolution. At 300 °C, the lower Na leach rates indicate some selective reprecipitation of Na (see above). In recent laboratory studies at

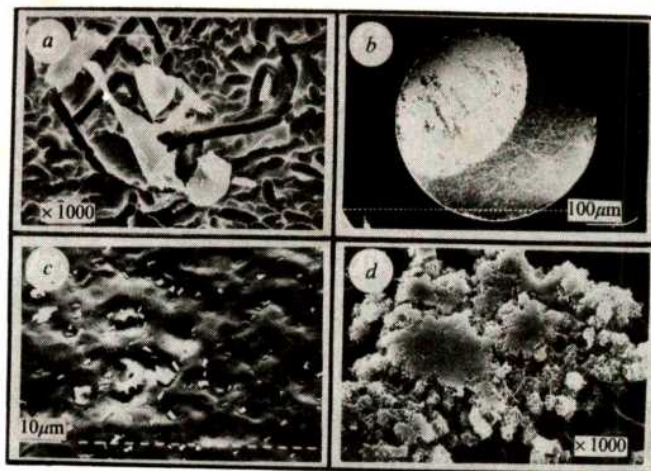


Fig. 1 Scanning electron micrographs of rhyolite glass after leaching with distilled water. a, Rhyolite at 200 °C, 3 days; b, rhyolite at 200 °C, 7 days; c, rhyolite + granite at 300 °C for 2 days; d, rhyolite at 300 °C, 3 days.

120 °C, Zielinski¹² has also found that Si, K, and U in rhyolites are leached congruently, but previous work on natural weathered rhyolites (perlites) has usually indicated preferential leaching of sodium^{6,7}. Also, recent work by White and Claassen¹³ on rhyolite leaching at 25 °C, shows diffusion controlled parabolic kinetics, rather than the linear kinetics expected for congruent dissolution^{8,9}. Thus the mechanism of leaching probably changes between 25 °C and our higher temperatures.

From the weight losses at 200 and 300 °C for rhyolite (Table 1), and assuming a first-order dissolution process, an activation energy of ~12 kcal is readily calculated. This is very similar to that reported by Adams¹⁴ for rhyolite leaching below 100 °C, and substantially smaller than for borosilicates.

The scanning electron micrographs of the leached 0.2 g samples showed that at 100 °C no noticeable pitting or etching occurs in either experiments (1) or (2). At 200 °C, rather deep etch pits appear after a few days (Fig. 1a), and a crystalline product begins to cover the disk (Fig. 1b). This same product is produced in greater amounts in the 300 °C runs (Fig. 1d) and has been identified as muscovite by powder X-ray diffraction. The stability of muscovite at these temperatures is not surprising from Meyer and Hemley's laboratory observations¹⁵.

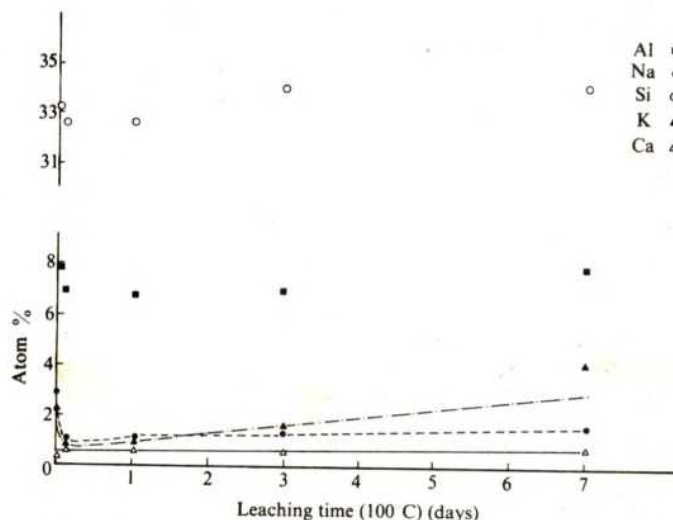


Fig. 2 ESCA surface analyses of the rhyolite disks leached in distilled water at 100 °C. The lines for Na, K and Ca are drawn only for clarity.

In contrast with the formation of large amounts of muscovite in distilled water, the glass disks reacted in the presence of granite (Fig. 1c) showed only a small amount of crystalline product which, based on the morphology of the crystals, appears to be feldspar. There must be a precipitation of aluminosilicates on the crystalline phases of the granite, which may be similar to the well-known amorphous silica reprecipitation¹⁶. This reaction is now under more detailed study. Our results, together with those of McCarthy *et al.*¹⁷, suggest that more work is needed on glass dissolution in the presence of complex rock matrices.

Table 2 Leach rates in g(glass) per cm⁻² per day⁻¹*

	T (°C)	Sample weight (g)	Leach rate	
			Weight loss	Na
Na borosilicates	100		$\geq 9 \times 10^{-5} \dagger$	—
			$3.5 \times 10^{-4} \ddagger$	—
Rhyolite	100	1.3	$3.6 \times 10^{-5} \S$	ND
		0.5	$3.3 \times 10^{-5} \S$	5.4×10^{-5}
	200	0.2	$1.7 \times 10^{-4} \S$	ND
		0.5	$4.2 \times 10^{-4} \S$	4.4×10^{-4}
		0.5	$3.7 \times 10^{-4} \S$	4.8×10^{-4}
	300	0.2	$1.3 \times 10^{-3} \S$	4.8×10^{-4}
		0.5	$1.3 \times 10^{-3} \S$	8.6×10^{-4}

* All leach rates are based on 1 week data. The Na leach rates were calculated assuming all of the glass constituents leached at the same rate as sodium (congruent dissolution).

† Ref. 5. ‡ Ref. 2. § From Table 1.

The ESCA and SIMS measurements complement the above observations, and show that rhyolite has hitherto unobserved surface leaching properties for a glass. From the areas of the appropriate ESCA peaks (Na KLL Auger, K 2p, Al 2p, Si 2p, Ca 2p and O 1s), the glass surface (≤ 50 Å) is readily analysed¹⁸⁻²⁰. The ESCA surface analyses of the unleached samples in atom. % are in semiquantitative agreement with the bulk probe analysis Bulk (and ESCA) analyses in atom. % are: Na; 2.8 (2.9); K; 2.0 (2.2); Al; 5.0 (7.9); Si; 26.5 (33.2); Ca; 0.2 (0.4); O; 63.3 (53.4). At 100 °C, Na and K are indeed leached preferentially from the surface (Fig. 2), as was indicated by the Na leach rates (Table 2). However, on further leaching, at 100 °C, the Na and K surface concentrations increase and approach the unleached values. Moreover, for all samples leached at 200 and 300 °C (1 hour to 1 week), all elemental surface concentrations are the same (within $\pm 10\%$) of the unleached sample. Congruent dissolution is the major mechanism of leaching in these experiments, as was indicated by the leach rates in Table 2.

Further confirmation of the congruent leaching mechanism comes from the SIMS measurements on unleached samples and samples leached at 100 and 300 °C. The leached samples show very similar concentration profiles to the unleached samples. All samples show very little concentration gradient for any of the elements measured; and in the unleached sample and the sample leached at 300 °C, Na and K are actually slightly enriched at the surface. All other SIMS measurements (ref. 8 and N. S. McIntyre, unpublished data) on other glasses have indicated a sharp decrease in surface alkali content after leaching.

We conclude that rhyolite is much more stable to leaching than are the borosilicates previously studied. Moreover, above 100 °C, the SEM, ESCA and SIMS results give no evidence for a surface depleted layer, as has been shown for many other glasses. Even without this layer, the glass is comparatively stable. Crystallisation of muscovite or clay minerals¹² will serve as useful sorbents for leached ions. More research is needed on such aluminosilicate glasses to understand fully the mechanism of dissolution and to minimise the temperature of formation while retaining their high leach stability.

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Winds in the thermosphere of the Northern Polar Cap

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A preliminary report is presented here of a sequence of measurements of the thermospheric wind in the Northern Polar Cap covering nine complete periods of 24 h. This unique data set covers all local times and a range of magnetic activities with K_p values from 0 to 6⁻. Certain major features of the wind pattern were found to be independent of magnetic activity: night-time winds were steady and approximately antisolar, whereas in the morning and evening sectors the wind weakened and the direction became variable as if the observation point passed through a vortex. On the dayside, the wind was northwestward in quiet conditions but tended towards the antisolar direction with increasing activity. The simplicity of the wind pattern in geomagnetic coordinates and its similarity to the published maps of ion drift strongly suggest that the momentum of the neutral gas is supplied from active ion drag and hence the energy of circulation comes from the magnetospheric electric field.

A ground-based optical Doppler technique was used and the observations were made at Longyearbyen (geographic coordinates 78.2° N, 15.6° E) during January 1979. At that time of the year there is unrelieved darkness for the whole day which permitted the continuous observation of the aurora. Although discrete forms were frequently seen in localised areas of the sky, for the most part, observations were being made on the diffuse background which was of subvisual intensity. Measurements of the oxygen emission at 630 nm in the aurora were used to find the wind velocity at a height of ~230 km. Using a passively stabilised scanning Fabry-Perot interferometer determinations of the horizontal components of the neutral wind were made with a typical error bar of 20 ms⁻¹. The maximum speed frequently occurred between 2100 and 0300 MLT (magnetic local time) and was directed towards the Equator. During these observations, the highest speed observed was 450 m s⁻¹ which

occurred at 2400 h on the 27 January 1979 when the magnetic character figure K_p was 4⁺. On less active periods the wind at 2400 h was smaller; for example, on 21 January 1979, when $K_p = 0$, it was 100 m s⁻¹.

In Fig. 1a, a 24-h plot in geomagnetic coordinates shows the wind vectors at 15-min intervals drawn with their tails on the circle which is the locus of the position of Longyearbyen as the Earth rotates. The predominance of the antisolar wind is evident except for a few hours near 0600 and 1700 h magnetic time when the wind weakens which suggests that there are local vortices in the vicinity. The major region of cross-polar flow seems to lie poleward of 78.2°N during the morning and afternoon periods. The value of the magnetic character figure A_p for this day was 23. The wind pattern for a less active day, 21 January 1979, when A_p was 11, is shown in Fig. 1b. Although the antisolar flow clearly dominates the nightside, it is mixed with a westward component on the dayside. This builds up from early morning, maximising in the afternoon and diminishing in early evening before the onset of the equatorward wind. Such behaviour is more typical of lower geomagnetic activity, and was also observed on the 9, 12, 20 and 22 January when A_p was 15, 10, 12, and 14 respectively. Other examples of the same type as in Fig. 1a were found on 24 and 25 January when A_p was 23 and 34 respectively.

Reports of rocket-borne experiments flown at high latitudes within the Polar Cap¹⁻³ have also shown, for the limited duration of the vapour trail, the existence of the antisolar wind. The occurrence of the westward wind on the dayside is a feature of less active days when one may expect that the auroral oval has

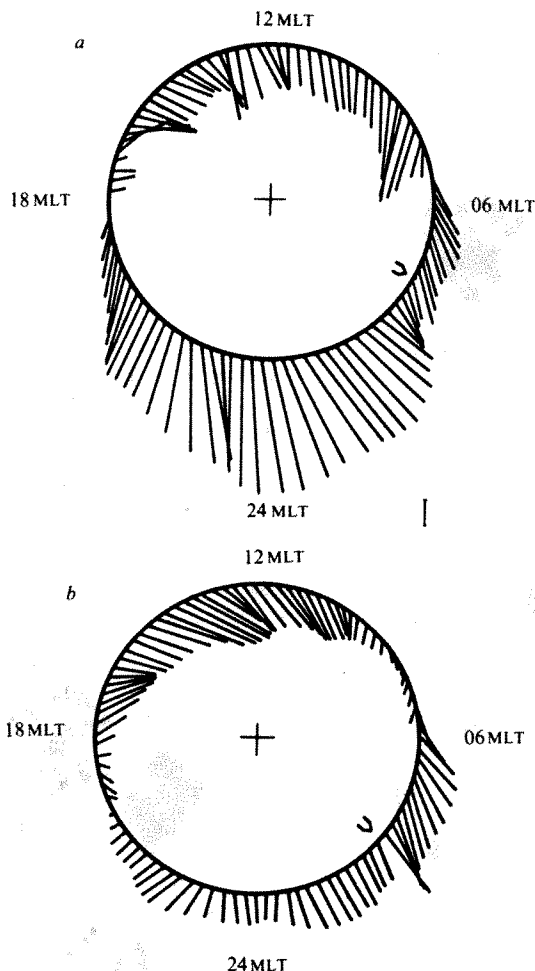


Fig. 1 A 24-h plot in geomagnetic coordinates showing vectors representing the neutral wind in the polar thermosphere during: a, 27 January 1979. b, 21 January 1979. The circle is at a geomagnetic latitude of 73.1°N, traced out by the observing site during the period of observation. The vectors are drawn with their tails on the circle. Scale bar, 100 m s⁻¹ MLT.

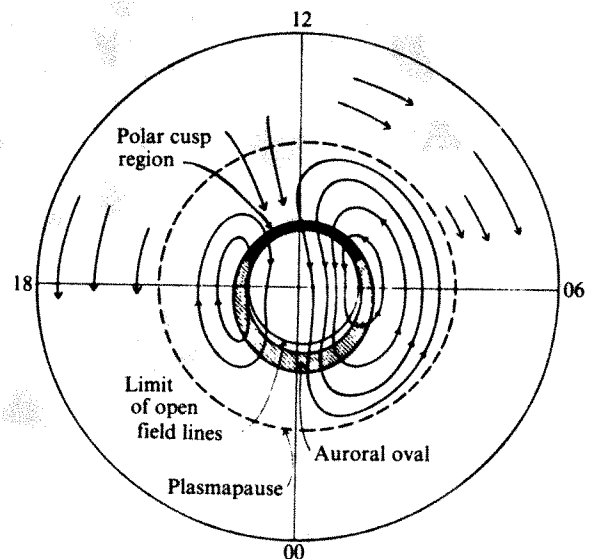


Fig. 2 A qualitative view of global winds including the effects of convection electric fields. Figure 13 from Fedder and Banks⁵ redrawn to make a direct comparison with Fig. 1.

contracted and the magnetospheric cusp lies on magnetic field lines to the north of Longyearbyen. In these conditions, the ionosphere above the observatory site is in a region of weak penetration of the magnetospheric electric field and, being in shadow from the solar EUV radiation, has a low ion concentration. The ion momentum source being weakened, the influence of the global scale pressure distribution may have greater significance. Note in this connection, that the geomagnetic and geographic frames of reference at Longyearbyen differ in azimuth by ~45°. In consequence, when the data are presented in geographic coordinates, these daytime winds are within a few degrees of westward. A possible mechanism, not involving ions, would require a large scale pressure gradient with pressure increasing towards the Pole which, combined with the Coriolis effect, would drive a westward wind. However, the 0G06 data on exospheric temperatures showed a broad temperature minimum at the Pole at the winter solstice⁴ which is not consistent with the present interpretation.

The general features of a high latitude neutral wind pattern dominated by ion convection are shown in Fig. 2 as given by Fedder and Banks⁵ and are also shown by the present results. The cross-polar antisolar flow arises from the dusk to dawn magnetospheric electric field. Vortices in the morning and evening occur where the sunward convection in the auroral zone causes the neutral wind to reverse direction as latitude decreases. Some workers conclude that winds at high latitudes up to the auroral zone are very much dependent on pressure gradients arising due to Joule heating⁶. However, the major features of the thermospheric wind in the Polar Cap seem to be consistent with the theory that most of the momentum is derived directly from the convecting ions⁷ which transfer energy stored in the magnetospheric electric field into kinetic energy of circulation by active Hall drag. The results presented here show that more energy is transferred with increasing magnetic activity and indicate that a greater latitudinal extent of the thermosphere is included in the circulation with greater A_p .

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Phytoplankton growth and zooplankton grazing in oligotrophic oceans

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Central oceanic regions such as the Sargasso Sea and the North Pacific Gyre have traditionally been thought of as biological deserts^{1,2}. New phytoplankton production estimates³ and interpretations of physiological data^{4,5} have suggested that these areas are, in fact, highly productive and that nutrient exchanges supporting these high growth rates are extremely transient. Fast phytoplankton growth rates must be balanced by equally fast zooplankton-caused mortality. Zooplankton abundances and their filtration rates, however, are consistent with slow phytoplankton growth rates, not fast. It is shown here that the dominant grazers in oligotrophic areas seem to be microzooplankton, and that molecular diffusion limits the effect of nutrient pulses, implying that bulk nutrient concentrations are most important. Present growth studies imply that phytoplankton can meet their growth needs with nanomolar ammonia concentrations.

Ambient concentrations of such nitrogenous nutrients as ammonia are below detection limits with conventional chemical methods in surface waters of central oceanic oligotrophic areas^{6,7}. Indirect evidence suggests that the concentrations of ammonia and urea in the North Pacific central gyre⁷ are less than 50 nM. This is far lower than average values for unpolluted coastal waters^{8,9}, and very low compared with half-saturation concentrations for most phytoplankton grown in cultures¹⁰.

Growth rates for phytoplankton growing with steady-state nutrient kinetics at such low concentrations would be low, a fact supported by ¹⁴C incubation measurements in the central North Pacific Gyre. Average specific growth rate, calculated as carbon assimilation rate divided by living carbon concentration⁷, is about 0.1 per day.

Phytoplankton growth measured by other techniques can yield much higher rates. The shortest doubling time reported is 3 h in the Sargasso Sea, calculated from ATP concentration changes in incubated water samples³. This is equivalent to a specific growth rate of 2.8 per day for a 12-h growth day.

Cellular nutrient status determines and can, therefore, indicate growth rate. Phytoplankton grown in cultures have their carbon, nitrogen and phosphorus contents in the same relative proportions as those in oceanic phytoplankton (in the Redfield ratio) only when growing at maximal rates⁵. If the physiology of oceanic phytoplankton is the same as that of

cultured phytoplankton then oceanic cells also grow at maximum rates⁵. Oceanic cells with the same maximum growth rate as cultured cells would grow at 2–3 per day.

These high growth rates cannot be supported for cells growing in a steady state with measured nitrogenous nutrient concentrations. Because slightly N-depleted phytoplankton display elevated uptake rates when compared with N-sufficient algae and because a source of regenerated nitrogen is zooplankton excretion, McCarthy and Goldman⁴ suggested that phytoplankton in oligotrophic oceans may obtain their nitrogen needs by rapid, transient uptake of excreted nitrogen. They noted such nutrients should be present at concentrations well above average in the wakes of swimming animals. They recognised that such elevated concentrations would persist only briefly. Thus, phytoplankton might appear to be growing successfully at the expense of virtually undetectable small-scale nutrient pulses.

The predominant source of phytoplankton mortality in low nutrient input regions such as mid-ocean gyres is zooplankton grazing. Calculation of grazing rates by multiplying typical laboratory-measured filtration rates and field-measured grazer densities (Table 1) shows that microzooplankton, especially protozoa, are dominant herbivores in the loss to grazers of 11% per day (0.11 per day) in the oligotrophic Pacific.

The conclusion that microzooplankton in the North Pacific Gyre are the dominant grazers explains the low rate of zooplankton excretion measured there¹¹. Specific regeneration rate of the macrozooplankton, calculated by dividing 2.4 nM per day nitrogen regeneration rate¹¹ by the 0.2 μM particulate nitrogen concentrations, is 0.012 per day. Microzooplankton feeding at a total filtration rate 5.4 times as fast (Table 1) will increase this rate to 0.007 per day.

Continuous, steady state uptake has specific growth rates equalling specific uptake rates. To achieve a 0.1 per day growth rate, concentrations less than now measurable would be adequate. For example, *Thalassiosira pseudonana* growth rate is 2.6 per day (81% of maximum) for ammonia concentrations at the detection limit, 0.1 μM (refs 22, 23). If growth follows the Monod equation,

$$\mu = \mu_{\max} \frac{C}{C + K_s} \quad (1)$$

with μ the specific growth rate, μ_{\max} the maximum growth rate, C the ammonia concentration, and K_s the half-saturation concentration, then a K_s value of 23.5 nM can be calculated from the above concentration and growth rate. Such a cell could maintain a growth rate of 0.1 per day at an ammonia concentration of 0.8 nM. A growth rate of 2.6 per day would, of course, require 0.1 μM ammonia.

Short term non-steady state ammonia uptake rates can be much faster than growth rates: instantaneous ammonia uptake rates are as high as 15 per day, equivalent to doubling times of 0.05 days or 4.3×10^3 s (ref. 4). Phytoplankton cells would have to take up nitrogen at maximum capacity for this length of time to accumulate enough nitrogen to support one cell division.

Table 1 Biological scales for central N. Pacific Gyre region

	Phytoplankton	Protozoa	Microzooplankton Nauplii	Post-nauplii	Macrozooplankton Copepods	Thaliaceans
Concentrations (C) (individuals per litre)	600,000*	655*	3*	1*	0.1†	$1 \times 10^{-4} \ddagger$
Separation distance (D) (cm)	0.1	1.1	5	10	21	—
Swimming speed (S) (m per day)	—	120§	—	—	1,200	—
Daily path separation (cm)	—	0.01	—	—	0.29	0
Individual filtration rates (ml per individual per day)	—	0.120¶	2#	10#	168**	500††
Total filtration rate (ml per litre per day)	—	75	6	10	16.8	0.05

Total filtration rate is 108 ml per litre per day, equal to a 0.11 per day grazing rate. Separation distance is $C^{-1/3}$. Daily path separation assumes all swimming paths are parallel and equally spaced, $(C^{-1}S^{-1})^{1/2}$.

* Ref. 17; † M. M. Mullin, personal communication; ‡ doliolids are dominant Thaliaceans (J. A. McGowan, personal communication); § ref. 18; || ref. 19; ¶ ref. 15; # ref. 20; ** ref. 21; †† Filtration rate is that of *Doliolletta gegenbaui* (D. Deibel, personal communication).

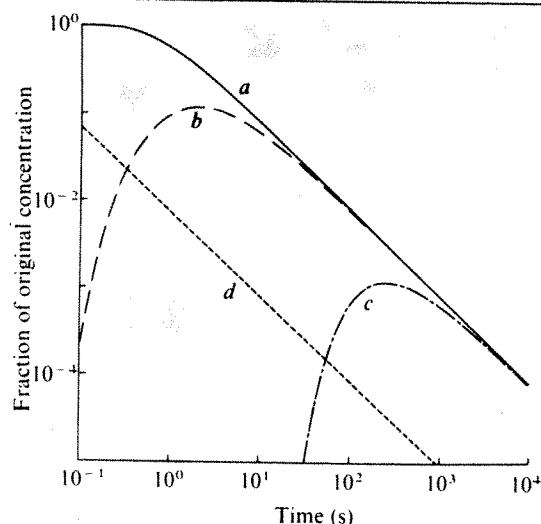


Fig. 1 Concentrations through time after a plume is created. Concentrations are fractions of original plume concentration. *a*, *b*, *c* show the effect of a 100 μm wide plume which is representative of a copepod's passage. *a*, At centre of plume; *b*, 100 μm from plume centre, 50 μm from initial plume edge; *c*, 1,000 μm from plume centre; *d*, effect of 10 μm wide plume representative of protozoa passage at centre of plume.

Phytoplankton growing at 0.1 per day could meet their nitrogen needs at uptake rates 1% of the maximum 15 per day.

A discontinuous nutrient source would be excreta from animals swimming near a phytoplankter. From the moment the animal excretes them, nutrients diffuse away. A phytoplankter will see a different range of nutrient concentrations depending on where it is relative to the plume. The concentration in a plume with square cross-section can be easily calculated. The relative concentration, f , at a given time after excretion, t , at a distance away from the centre along the centre line, x , for a plume of initial width, $2a$, and diffusivity, D ($= 10^{-5} \text{ cm}^2 \text{ s}^{-1}$),

$$f = \frac{1}{2} \left\{ \text{erf} \left(\frac{a-x}{2(Dt)^{1/2}} \right) + \text{erf} \left(\frac{a+x}{2(Dt)^{1/2}} \right) \right\} \times \text{erf} \left(\frac{a}{2(Dt)^{1/2}} \right) \quad (2)$$

where $\text{erf}(z)$ is the error function of z (ref. 12, p. 56).

The results (Fig. 1) show that even within the plume, nutrient concentrations drop rapidly. Three hundred seconds after a copepod-sized pulse (100 μm across) is released, concentrations have dropped by three orders of magnitude at the pulse centre and are a maximum 0.1 cm away that is $<0.001\%$ of the initial concentration. Molecular diffusion away from protozoan wake will be faster. Concentration at the centre of a 10 μm plume will have dropped to 0.1% of the initial concentration within 0.1 s. Because they will be further from the pulse most phytoplankton will see even lower concentrations.

Ammonia concentration in a plume behind a swimming herbivore can be calculated several ways. A grazer could be viewed as converting the particulate nitrogen in the water it filters to ammonia. By continually converting the 0.2 μM particulate nitrogen⁷ to ammonia, it would leave a trail of 0.2 μM ammonia. This supposes continual conversion. Sporadic excretion would raise concentration in a given pulse but decrease water volume affected. McCarthy and Goldman⁴ calculated that such a sporadic pulse could contain 5 μM ammonia around a copepod. Thus, the maximum nutrient concentration behind a protozoan will have decreased from 200 to 2 nM within 1 s; the same concentration change behind a copepod would occur within 100 s. These times are much shorter than the 4,300 s earlier calculated as the length of time required to accumulate sufficient N for a doubling. Individual nutrient pulses are not sufficient to be the main nitrogen source for phytoplankton.

These calculations neglect concentration decreases caused by nutrient uptake and assume that only molecular diffusion is important. Phytoplankton and turbulence cause concentrations to decrease faster than has been calculated here.

Usefulness of these molecular diffusion calculations depends on nutrient recycling occurring on small scales. Comparison of feeding rates for potential oceanic herbivores shows that small grazers—protozoa and copepods—dominate the larger thaliaceans, accounting for 99% of water filtered. The marine copepod *Paracalanus parvus* incorporates 37% of ingested particulate nitrogen and recycles the rest¹³. If mid-oceanic grazers recycle at the same 63%, then microzooplankton and copepods recycle at least 62% of ingested primary production for uptake by surviving phytoplankton, either directly or through bacteria. Thus, nitrogen cycling occurs predominantly on the microscale. Inputs on a larger scale, from salp aggregates or fish schools, may be more visible but less important.

Importance of nutrient interactions on a small scale is analytically convenient because diffusion-based mixing processes are so much better understood than those based on turbulence. Additionally, the complete range of phytoplankton-herbivore interactions would be occurring at any given time in the medium scale water sample. This would imply that the mean nutrient concentration through time at a given phytoplankter (that is, what a cell sees) would be the same as the mean concentration through space at a given time (that is, a water sample). A linear uptake-ammonia relation would imply that mean nutrient uptake rate is linearly related to the mean concentration through time at the cell, which is in turn linearly related to the measured concentration. As long as uptake rate is a linear function of ammonia concentration, the amount of ammonia will provide a useful mirror of an individual phytoplankter's environment. Less than linear uptake rates, indicative of saturation of cellular systems, would cause a linear model to overestimate cellular nutritional status.

Microzooplankton, which control N cycling in the temporally and spatially uniform central North Pacific Gyre¹⁴, need not dominate elsewhere: in the very heterogeneous coastal California protozoa are relatively unimportant¹⁵. The northern Sargasso Sea, where cold core rings introduce variability not seen in the central Pacific¹⁶, may have different grazers. However, similarity in zooplankton volumes for the North Pacific and the North Sargasso Sea^{14,16} suggest that the two oligotrophic systems are similar.

Why, then, do oceanic phytoplankton show nutritional composition characteristic of nutritionally fit cells? If oceanic algae grow at a rate of 0.1 per day and if the relationship between cellular nutrient status and maximum growth rates found in culture studies⁵ apply to all phytoplankton, then algae from oligotrophic oceanic areas have lower maximum growth rates. This would imply that the algae have adapted to perpetually low growth rates by losing the ability to grow fast. Further understanding of nutrient cycling in these oligotrophic areas will depend on the development of techniques to measure these low concentrations.

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Stromatolites 3,400-Myr old from the Archean of Western Australia

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Internally laminated conical mounds characterise a regionally extensive chert unit near the top of the 3,400-Myr old Warrawoona Group in the Pilbara Block of Western Australia. The chert formed by silicification of a carbonate-evaporite sequence deposited in shallow subtidal to intertidal environments. The morphology and internal organisation of the mounds described here suggests that they are conical stromatolites similar but not identical to members of the common Proterozoic group *Conophyton* Maslov.

The Warrawoona Group of the eastern Pilbara region of Western Australia (Fig. 1) is a typical Archean greenstone belt volcanic sequence^{1,2}. The 10-km thick section consists largely of volcanic rocks interstratified with cherty sedimentary units generally <30 m thick. Low greenschist-grade metamorphism has affected the entire sequence, but, in the areas studied, shearing is largely absent. Cherty units commonly show only minor recrystallisation.

During sedimentological studies in the eastern Pilbara, I identified a distinctive stromatolitic chert unit near the top of the Warrawoona Group in at least three of the Pilbara structural belts (Fig. 1). Here this unit is called the Strelley Pool chert for outcrops at Strelley Pool (21°06'33"S, 119°08'14"E) in the Pilgangoora Syncline (Fig. 1). The unit averages a relatively constant 20-25 m in thickness, although it reaches 50 m on the

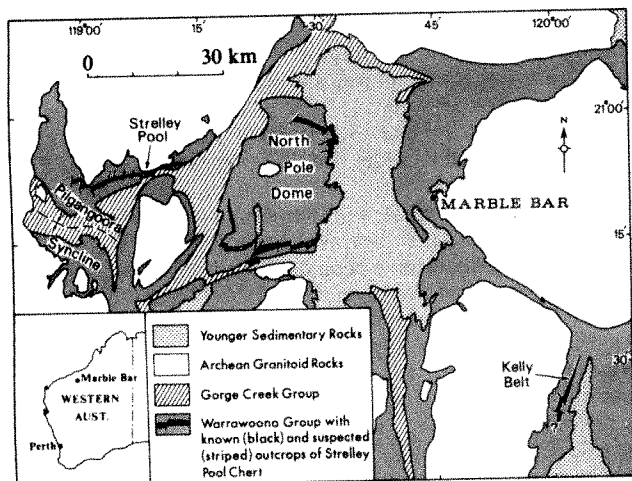


Fig. 1 General geological map of the northeastern Pilbara region showing known and suspected outcrops of the Strelley Pool chert.

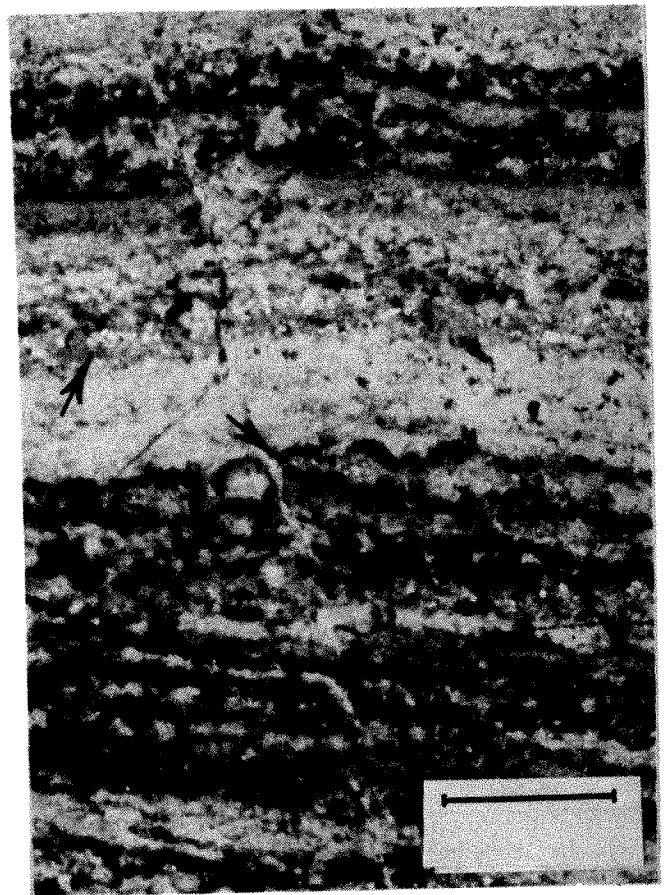


Fig. 2 Photomicrograph of alternating light and dark flat laminae from area between conical stromatolites. Arrows point to layers containing detrital quartz grains. Micronodular character of translucent laminae resembles fenestral texture of modern algal mats. Scale bar, 0.5 cm.

north flank of the North Pole Dome (21°00'43"S, 119°28'52"E). An excellent section in the Kelly Belt occurs near Spinaway Creek at 21°35'S, 120°01'01"E.

The Strelley Pool chert displays a nearly constant internal stratigraphy in the three structural belts in which it was identified. It can be subdivided into two main members. The lower 10-15 m consist largely of finely laminated chert. The rock shows thin, varve-like alternating laminae of light grey chert and dark grey carbonaceous and pyritic chert or of calcareous and non-calcareous chert (Fig. 2). The laminae may be flat and even but commonly form isolated or clustered conical mounds here interpreted to be stromatolites. This subdivision characteristically includes units up to 1 m thick composed of massive, coarsely crystalline silicified evaporite. These units are present in every section examined. In the Pilgangoora Syncline, a quartzite layer is present locally at the base of the chert, and thin laminae and wisps of detrital quartz occur irregularly in the overlying laminated chert (Fig. 2). Along at least two horizons, underlying laminae are truncated and cut by desiccation cracks filled with detrital sand, but, overall, coarse clastic layers, current structures, and evidence of exposure are rare. This subdivision was deposited in a subtidal to lower intertidal environment and originally consisted of interlayered evaporite and carbonate sediments.

The upper part of the Strelley Pool chert includes a variety of dark to light grey, white, and greenish cherts. Layers of large silicified evaporite crystals occur towards the base. Large pods of silicified collapse breccia and cavities containing silicified stalactitic and stalagmitic fill indicate the existence of considerably greater amounts of original evaporite and extensive post-depositional exposure, leaching, and solution collapse. Small intraformational conglomerates, scours, and other features

suggest deposition in low energy environments ranging from shallow subtidal to intertidal. In the Pilgangoora Syncline, this unit is capped by detrital volcanoclastic material that may represent alluvial deposits.

The regional consistency of the detailed internal stratigraphy of the Strelley Pool chert across the entire 100+ km extent of its known outcrop (Fig. 1); its distinctive and regionally consistent sedimentology; and the characteristic development of stromatolites are unlike other chert units in the Warrawoona Group and indicate that the Strelley Pool chert is not correlative with better known chert units at Marble Bar and near the base of the North Pole stratigraphic sequence¹. These cherts lie several kilometres stratigraphically below the Strelley Pool chert. The latter represents a single, large, regionally extensive Archean evaporite basin which covered much of the area of the modern eastern Pilbara.

Unbranched, conical, columnar stromatolites occur within the lower subdivision of the Strelley Pool chert across its entire outcrop. They occur as distinct conical mounds with a relief generally between 2 and 6 cm (Figs 3 and 4). Individual mounds may extend vertically through as much as 0.6 m of rock. Apical angles average 70° to 80° (Fig. 4), but are locally as low as 30°. The internal lamination is fine and continuous, even across areas between widely spaced stromatolites. In horizontal sections and rare occurrences where the stromatolites show in relief on bedding surfaces, their shape varies from circular to elliptical (Fig. 3).

These structures are considered to be biogenic for several reasons. Their gross morphology and internal structuring are like those of post-Archean conical stromatolites, particularly members of the group *Conophyton* Maslov^{3,4}. Fine laminae showing alternating light and dark layers are characteristic of many Proterozoic and early Archean unbranched conical columnar stromatolites. However, the Strelley Pool stroma-



Fig. 3 Surface morphology of conical stromatolites in the Strelley Pool chert. Note elliptical shape of many of the larger cones. This specimen from outcrops west of Strelley Pool at 21°08'S, 119°00'28"E. Scale bar, 5 cm.



Fig. 4 Vertical slab through same block of stromatolitic chert shown in Fig. 3. Note slight axial thickening of laminae. Darker areas are calcareous; light areas are nearly pure chert.

tolites differ from true *Conophyton* Maslov in generally lacking a well defined axial zone. Individual laminae instead are continuous across the apical region, showing a slight thickening in most specimens (Fig. 4). Their occurrence in shallow subtidal evaporitic deposits in the Pilbara accords well with previous interpretations that conical stromatolites commonly develop in subtidal environments by alternating periods of organic growth and chemical precipitation^{5,6}. The continuity of the laminae from stromatolite mounds to flat inter-mound areas in the Strelley Pool chert indicates that large areas of the sea floor were covered by flat organic mats. In the Pilgangoora Syncline, the flat cryptogalaminites contain detrital sand grains and sand laminae (Fig. 2), features characteristic of both modern and ancient algal and bacterial mats reflecting their binding and trapping ability.

Lavas from the Duffer Formation near the middle of the Warrawoona Group have been dated at about 3,450–3,490 Myr BP (refs 8, 9). These rocks are 1–2 km stratigraphically below the Strelley Pool chert, and, although the possibility of intervening unconformities cannot be dismissed, the overall igneous and sedimentological continuity of the Group suggests that major hiatuses are absent. Following deposition of the uppermost part of the Warrawoona Group and the suprajacent Gorge Creek sediments, the rocks were extensively deformed and intruded by granitoid plutons. The main period of deformation and associated plutonism has been dated at about 2,900–3,100 Myr (refs 10, 11). The possible age range for the stromatolites is, therefore, 3,450–3,100 Myr, but their close association with the older rocks suggests deposition at ~3,400 Myr.

The importance of the stromatolites in the Strelley Pool chert lies in their implications towards the nature of early life forms. Archean stromatolites have been described previously from the 2,600–2,700 Myr-old rocks in Canada^{12,13}, from the 2,500–2,800 Myr-old Bulawayan Group in Rhodesia^{14,15}, and from the 3,000 Myr-old Pongola Supergroup in South Africa¹⁶. Dunlop and others¹⁷ have previously reported oncolitic and mat-like structures which they interpret to be stromatolites from the Warrawoona Group, but they are apparently of local occurrence, do not show a well developed mound-like habit, and have not been documented as being biogenic.

The Strelley Pool stromatolites are similar in morphology and internal structure to stromatolites from younger Archean and Proterozoic sequences. If these and other Archean stromatolites

represent, at least in part, the activities of blue-green algae, as seem probable based on comparisons with modern stromatolites¹⁸, including *Conophyton*¹⁹, they would substantiate suggestions that photosynthesis was an effective source of oxygen 3,500 Myr ago²⁰. They also imply that the origin of terrestrial life lies much further back in geological time than 3,500 Myr.

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Stromatolites 3,400-3,500 Myr old from the North Pole area, Western Australia

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Stromatolites are the least controversial evidence of early life; they are organosedimentary structures resulting from the growth and metabolic activity of microorganisms¹. Before this report, however, the oldest well established occurrence was in the 2,900-3,000 Myr Pongola Supergroup of South Africa²; five or six additional occurrences are known from the later Archean³. The only proposed example from older rocks is of a possibly stromatolitic microfabric from 3,500 Myr cherts in South Africa⁴; as yet that interpretation has not been supported by the discovery of macroscopic stromatolites. Here we describe stromatolites 3,400-3,500-Myr old from the Pilbara Block of Western Australia. These are the oldest firmly established biogenic deposits now known from the geological record.

Only in rocks younger than 2,300 Myr, recording the second half of Earth history, is our understanding of the course of biological evolution closely constrained by palaeontological, geochemical and sedimentary evidence^{5,6}. Objects interpreted as microfossils have been reported from 3,300-3,500 Myr cherts from Australia⁷ and Africa^{4,8,9}, and 3,770 Myr quartzites from Greenland^{10,11}, but none has yet found general acceptance¹²⁻¹⁴. Sulphur and carbon isotope data have been interpreted as indicating that the origin of microbial sulphate-reduction occurred between 2,700 and 3,300 Myr ago¹⁵⁻¹⁷ and that photosynthesising microorganisms may have originated even earlier than the deposition of the oldest known sediments

(3,770 Myr)¹⁸, but as yet relevant data are few and at least in the example of the carbon isotopes are subject to differing interpretations¹⁹. Kerogen is abundant in some Archean sedimentary rocks²⁰, but the interpretation of this is complicated by the fact that carbonaceous substances can be produced abiogenically²¹, as well as by heterotrophic and autotrophic organisms.

Structures resembling stromatolites are abundant in a chert-barite unit in the dominantly ultramafic-mafic metavolcanic Warrawoona Group of the eastern Pilbara Block (Fig. 1). Many of these structures seem to have formed by abiological processes (such as slump folding and silica deposition in voids), and others, while possibly biogenic, are not distinctive enough to be interpreted unambiguously. Earlier studies recorded the presence of possible stromatolitic fabrics²², clasts possibly formed by desiccation of microbial mats (some having oncolite-like overgrowths)⁷ and possible stromatolites²³. The structure we describe here has a relatively complex morphology which provides more criteria for biogenicity.

The chert-barite unit at North Pole consists of bedded shallow water metasediments 40 m thick that persist for at least 30 km along strike^{24,25}. The maximum metamorphic grade is in the lower greenschist facies, and the rocks have been subjected to little strain (ref. 26, and B. James, personal communication). Four sediment types are present: (1) those interpreted as silicified detrital carbonate rocks, often containing relict grains of carbonate and small (up to 1 cm) silica pseudomorphs after gypsum; (2) stratiform barite, some of which can be shown to have replaced evaporative gypsum^{24,25}; (3) silicified mafic volcanoclastic rocks; and (4) those interpreted as primary siliceous sediments, now cherts. A model lead age of 3,420 Myr has been obtained from galena within remobilised barite at this locality (J. Richards, personal communication). The volcanic Duffer

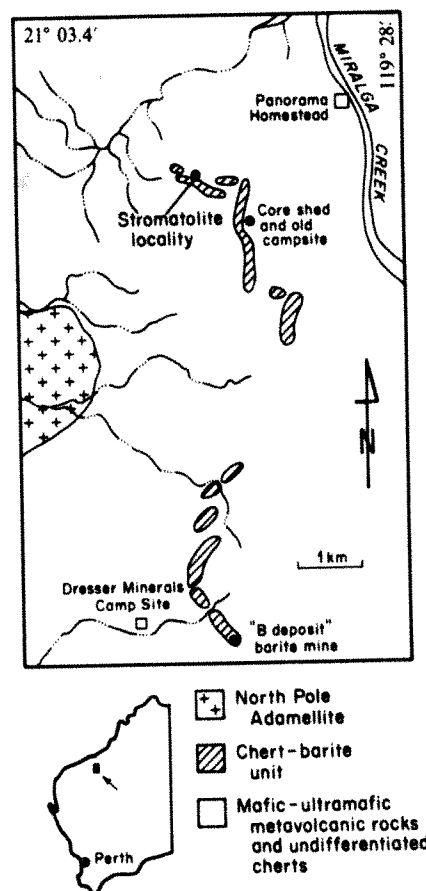


Fig. 1 Map of the northeastern part of the North Pole Dome, eastern Pilbara Block, showing the locality from which the stromatolite was collected. All rock units, apart from the post-tectonic intrusive North Pole Adamellite, are within the Warrawoona Group.

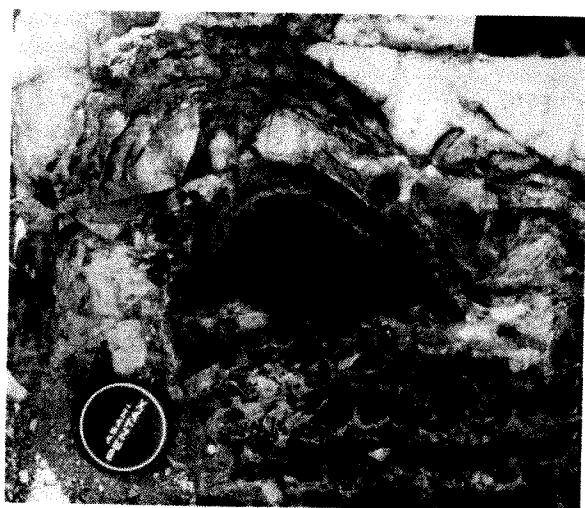


Fig. 2 Field photograph showing the exposed nodular and stratiform portions of the stromatolite. Scale, ~5 cm.

Formation, also within the Warrawoona Group and considered an approximate stratigraphic equivalent of the chert-barite unit²⁶, has yielded zircons that have been dated at $3,452 \pm 16$ Myr (ref. 27), and volcanics lower in the Warrawoona Group have recently been dated at $3,520 \pm 60$ Myr using Sm-Nd (P. J. Hamilton, personal communication).

The stromatolite described here is composed of dolomitic chert and was collected 5 m from the top of a 10.5-m thick exposure of bedded chert in an area of complex faulting 2.5 km WSW of Panorama Homestead (Fig. 1). Discussion of the possible original mineralic composition of the stromatolite is beyond the scope of the present paper. The bed underlying the stromatolite is composed of bands of clear to white chert, white agate, and red finely laminated jaspilite. The bands are separated by laminae of 1–2 mm wide rhombs of ankeritic dolomite. In outcrop, a single nodular structure 20-cm high by 25-cm wide (Fig. 2) was the only domical stromatolite visible; no similar structures were recognised in the same bed. The nodule protruded upward from a 20-cm thick bed of wavy laminated chert with lenses of intraclast and possibly ooid grainstone, into thin-bedded dolomitic chert. A specimen $60 \times 60 \times 40$ cm containing the nodule was collected and cut into slabs about 5-cm thick. This revealed several smaller, wavy laminated nodular stromatolites and a small escarpment encrusted by fine wavy laminae (Fig. 3).

It is apparent that the nodular and wavy laminated forms were primary sedimentary structures with relief, because undeformed overlying and adjacent beds wedge out against, abut against, or thin over the structures, and an erosional scarp has been encrusted by subsequently deposited laminae. The intraclasts found alongside the nodular structures are of two types: the first is thick (3–5 mm), flat, and similar in microstructure to the thin-bedded unlaminated cherts interbedded with the stromatolites; the second is thin (~1 mm) and arcuate to indulose, and indistinguishable in microstructure from the stromatolite laminae. These latter delicate clasts probably could not have survived long transportation, and probably were derived locally from the accreting stromatolite subsequent to desiccation.

The stromatolites have a banded microstructure²⁸ with wavy and wrinkled laminae predominantly 50–200 μ m thick, although many laminae are as thin as 20 μ m (Fig. 4). Domical laminar structures up to 3 cm wide occur frequently within the stromatolites (Fig. 3), and many persist vertically for more than a centimetre, producing pseudocolumnar structures. Many of the ooidal structures are flat-topped, with sharply deflexed margins. Junctions between contiguous domes are V-shaped. Small, discrete columns occur rarely within the large stromatolite nodule. These laminar structures are closely comparable with those of younger stromatolites²⁹.

There are abiogenic structures that resemble stromatolites²⁹, but of all such structures, only one class seems to provide a plausible alternative to the biogenic interpretation of the North Pole structures. That is the possibility that they may be deposits from splashing water, such as the coniatolites described from the Persian Gulf³⁰. However, such an interpretation is not consistent with the abundant occurrence of intraclasts having the same microstructure as the stromatolite (a feature absent from coniatolites because they are not susceptible to desiccation due to their compact, hard nature), or with the absence of dripstone structures, abundant coated grains, and microreticulate ridges on lamina surfaces, features characteristic of coniatolites³⁰. We conclude that the structures are correctly identified as stromatolites. Research on the recognition and distribution of stromatolites in the Warrawoona Group and on the palaeoenvironmental setting, is continuing (R.B. and J.S.R.D.).

It seems that there was a benthic microbiota 3,400–3,500 Myr ago, but its biological affinities are unknown. The common opinion in the literature is that the presence of stromatolites establishes the former presence of cyanobacteria, but that is an unwarranted interpretation, especially for Archean stromatolites^{3,31}. There are living examples of bacterial stromatolites built by other than cyanobacteria, for example, by *Chloroflexus*, a green, photosynthetic, filamentous bacterium which presently constructs stromatolites in hot springs^{31–33}. Furthermore, it is reasonable to suggest that there was a time, before the advent of the appropriate cyanobacteria, when the dominant stromatolite-builders were organisms such as *Chloroflexus*. This is significant because among the bacteria only the cyanobacteria release oxygen during photosynthesis; so at

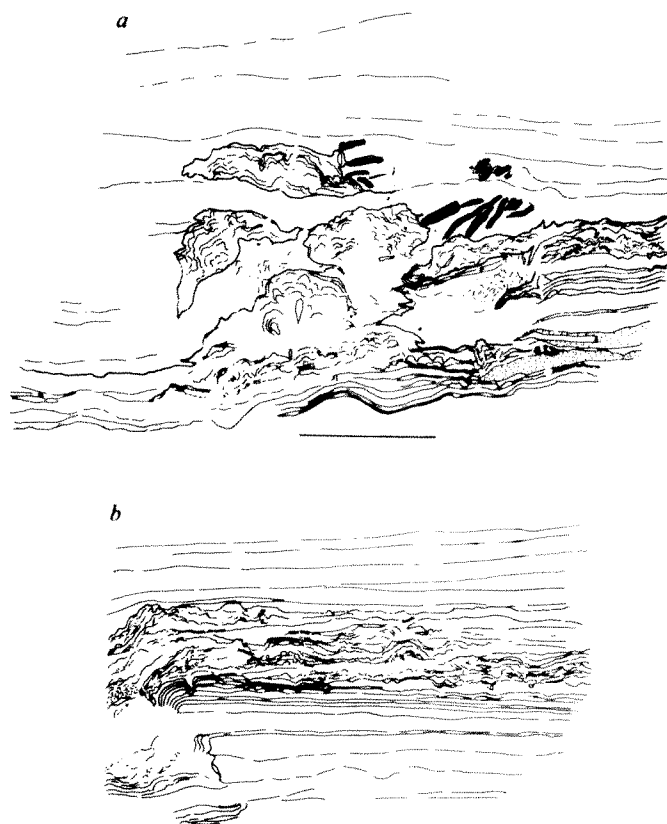


Fig. 3 Tracings of laminae revealed on sawn slab faces 2ii (parallel to and 13 cm further into the rock from the plane shown in Fig. 2) and A (at 90° to plane of a). The laminae consist of dolomitic chert. Note the three domical structures in the centre of a and the encrusted scarp to the lower left and the pseudocolumnar structures to the central left of b. The stipples represent possible ooid grainstones, the small dashes are intraclasts derived from the stromatolite and the large black tabular bodies are intraclasts derived from the overlying clear to white thinly bedded dolomitic chert. Scale bars, 10 cm.



Fig. 4 Photomicrograph in transmitted plain light of fine (20–100 μm) stromatolitic laminae composed of ferruginous dolomitic chert. Scale bar, 5 mm.

least for these very ancient rocks it is not possible to equate the presence of stromatolites with the former presence of oxygen-releasing photosynthesizers.

No microfossils have been found within the stromatolite, but a consideration of the microstructure allows us to draw some inferences about the morphology of the constructing organisms. As many laminae are as thin as 20 μm , these organisms must have been no larger than that, at least in one dimension. The regular, even, lamination of the stromatolite is comparable with that demonstrably produced by filamentous organisms in other stromatolites, and unlike that produced by coccoid organisms³⁴.

The microbiota seems to have inhabited an environment where it was intermittently exposed to the atmosphere and so inevitably was exposed to UV radiation, severe fluctuations in water potential³⁵, and diurnal temperature changes. Its presence may indicate that a protective ozone screen already existed. The microbiota had developed some strategy for coping with the danger of burial by sediment, which led to the accretion of the stromatolite, but it is not yet possible to say whether the strategy involved phototaxis, as in many younger stromatolites. Already at this very early stage in Earth history, a community of organisms existed that was adapted to life in an adverse, fluctuating environment.

The stromatolite described here was found by J.S.R.D. in 1977 during research for a doctoral project on the North Pole barite deposits. We thank the Bureau of Mineral Resources Geology and Geophysics, Australia, and the Geological Survey of Western Australia, for providing support in the field, and NASA Grant NSG 7489 and the NSF Waterman Foundation Award to J. W. Schopf for financial support. M.R.W. is on leave from the Bureau of Mineral Resources, Geology and Geophysics. J. M. Hayes, H. J. Hofmann, D. Gregg and L. Bettenay assisted with the collection of the specimen on which this study is based. We thank D. I. Groves for supervision and support, and J. W. Schopf, J. M. Hayes and M. Schidlowski for helpful comments.

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How do fish break the speed limit?

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Many studies have shown that tail beat frequency of teleost fish is closely related to their swimming speed. These findings were developed further by Wardle¹, who showed that maximum tail beat frequency and thus swimming speed could be predicted by measuring the twitch contraction time of the fast swimming muscles. The contraction time increases with the length of the fish and decreases with increase of temperature. Several authors have observed and recorded swimming speeds greater than the predicted speed limit. For example, Stevens and Neil² report that skipjack tuna (*Katsuwonus pelamis* (L), length (L) 0.5 m) could swim at a speed of 10 m s⁻¹ at 32 °C, whereas the maximum predicted speed (extrapolating Wardle's¹ data) was only 8 m s⁻¹. Walters and Fiersteine³ recorded a yellowfin tuna (*Thunnus albacores* (Bonnatare), L = 0.98 m) moving at 20.8 m s⁻¹ and Wahoo (*Acanthocybium solandrin* (Cuvier), L = 1.13 m) at 21.4 m s⁻¹. These two records show just twice the predicted maximum swimming speeds. Unfortunately no observations of the tail beat frequency were made during these high-speed swim records. Brill and Dizon⁴ have shown that five skipjack tuna (L 0.37–0.43 m) had muscle contraction times similar to non-scombroid teleost species of this size measured by Wardle¹. It seems therefore that the tuna family (Scombridae) do not have faster maximum tail beat frequency than other teleosts of the same size and temperature. We show here how this paradox can be solved by the fish using a different swimming style, requiring greater power and efficient interaction between the propelling surfaces and the water, but allowing the fish to move twice as far for each tail beat.

The normal relationship between tail beat frequency and speed can be explained by a simple analysis of the fish's swimming movements. We have analysed² the lateral body movements of cod, *Gadus morhua*, swimming at constant speeds and defined a two-wave system which allowed exact description of swimming movements in most teleost fish. Fish move forwards by means of a series of waves of contraction of the lateral muscles on alternate sides, starting in the myotomes behind the head and ending where the last myotome inserts on the tail. A wave causes lateral displacement of body and fins and these in turn cause forward motion by reaction with the water. During constant speed swimming the speed (v) of the wave moving from head to tail is always slightly greater than the forward swimming speed (u); so that u/v is less than 1. The wave on the body is S-shaped when seen from above and has a measurable wavelength λ_b . The amplitude of the wave is small in the head region and greatest at the tail tip where it is normally 0.2 times body length. The track traced by the tail tip through the water forms a second wave with wavelength λ_s , which is always shorter during constant speed swimming than λ_b , but has the same period (T); λ_s can be much longer or much shorter than λ_b , depending on whether the fish is slowing down or accelerating. It is easy to measure λ_s but measurement of λ_b and its velocity (v) requires accurate identity and location of the wave crests on images of the swimming fish. Towards the head, the amplitude of the wave can be very small and the wave crests difficult to identify. New techniques for filming and accurate analysis enabled us to measure the wave parameters and development of the relationship between the two wave systems discussed before⁵. We confirmed that $u/v = \lambda_s/\lambda_b$. Previous studies have shown that λ_s , which is equal to the distance moved forwards after completion of one tail beat cycle or the passage of one wave, is near to 0.7 times body length (L). For all the species studied, including goldfish (*Carassius auratus*) and tuna, this figure holds, and tail beat frequency gives a straight line when plotted against swimming speed (measured in lengths per second) during constant speed swimming and over a wide range of speeds⁶⁻⁹.

With u/v always slightly less than 1, λ_s always close to 0.7 L and λ_b always longer than λ_s (in cod $\lambda_b = 0.85 L$, ref. 4) it becomes evident that there is relatively little variation in the relation between λ_b and L . All those species where tail beat frequency has been related to constant swimming speeds have therefore shown slightly more than 1 λ_b within the straight body length (L).

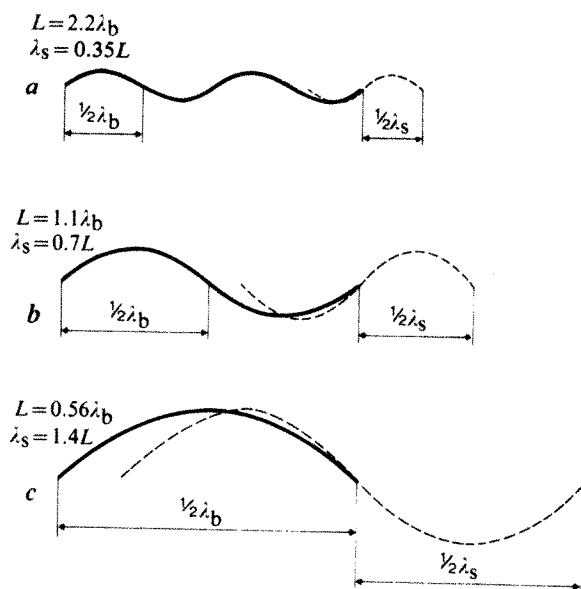


Fig. 1 Firm lines represent the wave (wavelength λ_b) on the body of a fish with straight length L . The dashed lines show the track of the tail tip (wavelength λ_s) after one completed tail beat. Note the distance moved forwards after two contractions of the lateral swimming muscles: a, 0.35 L ; b, 0.7 L ; c, 1.4 L .

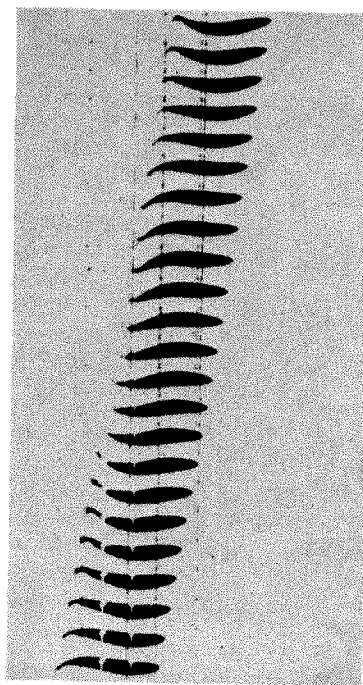


Fig. 2 Mackerel *Scomber scombrus* L., length 0.345 m, speed 2.9 m s^{-1} , swimming in the style shown in Fig. 1b, printed from 16-mm cine film made at 200 frames s^{-1} (Locam camera). Fish were illuminated with 150-W tungsten lamp, the light source guided by fibre optics to shine from points beside the camera lens to create silhouettes of the fish moving across a marked screen of reflex reflector material (3M high intensity Scotchlite). The reference lines are 25 cm apart. The tail sweeps from left to right in 35–40 ms, a time close to the twitch contraction time at 12°C .

To develop this important point let us compare the normal fish discussed so far with one fish showing two complete waves on its body so that $L \approx 2.2 \lambda_b$ and another fish of the same length but with only half a wavelength on its body so that $L \approx 0.56 \lambda_b$ (Fig. 1). Consider for each case the result of completing one tail beat cycle during constant speed swimming in time T . The track of the tail tip in each case is indicated by the dashed lines, and the distance moved forwards is a different λ_s in each case. In Fig. 1a the fish moves forwards less than 0.35 L . In the normal fish represented by Fig. 1b the forward movement is 0.7 L . In Fig. 1c, showing the fish with $L \approx \frac{1}{2} \lambda_b$, the distance covered is about 1.4 L . That is exactly twice the progress of the normal fish in the same time T . In all three cases the distance moved is the result of one contraction of the right and one contraction of the left lateral swimming muscles. This means that the shortest time in which each of the illustrated manoeuvres (Figs 1a–c) could be completed is twice the twitch contraction time as discussed by Wardle¹. This argument suggests that if L could ever approach or even equal $\frac{1}{2} \lambda_b$, the fish could bound forwards at up to twice the speed of the normal fish using the same contraction time. However, consider the problems of swimming at maximum speed with $L = \frac{1}{2} \lambda_b$. It is reasonable to suppose that normal fish swimming at maximum speed are contracting nearly the whole of the fast, white anaerobic muscle in each myotome in order to develop sufficient force in the wave to counteract the drag of the water on the fast moving body. This force (F) increases with the swimming speed to the power 1.8 where $F = \frac{1}{2} \rho A u^2 1.2 C_f$ (ref. 10). The flow around fish longer than 0.50 m swimming at 5 m s^{-1} at 30°C will be turbulent and C_f , the drag coefficient, will be proportional to $\text{Re}^{-0.2}$ and so proportional to $u^{-0.2}$. F is then proportional to $u^{1.8}$. (Re is Reynolds number, ρ is the density of water and A the body surface area.) The power (Fu) increases with $u^{2.8}$. That means to double the speed, seven times the power is required; equivalent to seven times the volume of swimming muscle. A fish swimming with $\frac{1}{2} \lambda_b = L$ might reason-

ably be expected to have a body diameter three to six times greater than a fish of the same actual length but with $L = \lambda_b$. However, a greater length of each lateral muscle will be involved in developing the movement when $L = \frac{1}{2}\lambda_b$, so this could be an overestimate. The increased muscle power will require extra strengthening of the spinal column and the surfaces which propel the fish must also be able to shed effectively seven times the power. Power consumption has been measured in cod (length 0.73 m) swimming at 3 m s⁻¹ at about 30 W kg⁻¹, and an estimated 100 W kg⁻¹ was required to swim at its maximum speed of 5.5 m s⁻¹ (ref. 10). A cod-like fish swimming at twice this speed might need to dissipate up to 700 W kg⁻¹. The thick body, strong spinal column and stiff high tail blade of the Scombridae does satisfy to some degree these points.

Is there real evidence that fish can swim with a body wavelength longer than the body length? A graph by Magnuson, summarising the relationship between tail beat frequency and swimming speed in the Scombroidei (fig. 16 in ref. 11), shows a series of measurements where at slower swimming speeds the distance moved forwards, λ_s , for completion of one tail beat is greater than one fish length (L). However, as the speed increases λ_s becomes less than 1 L and all the other scombrid data presented in the figure indicate λ_s similar to other teleosts at

about 0.7 L . It is possible that where λ_s was found to be greater than 1 L , the fish were slowing down. Preliminary measurements of films we have made show that the mackerel *Scomber scombrus* L. (Scombroidei) (lengths 0.33 and 0.345 m) swim with a complete 'S' wave on the body (Fig. 2) at 1.17 and 2.9 m s⁻¹ with $u/v = 0.86$, $\lambda_s = 0.76L$ and $\lambda_b = 0.89L$, all parameters similar to those for the cod. The cine film frames (Fig. 2) show the larger fish swimming at 2.9 m s⁻¹, using the swimming muscles close to their twitch shortening time (40 ms, 9°C) with a tail beat frequency of 11 Hz. The observed high speed swimming of some fish species does exceed the speed limit set by current knowledge of fish swimming, which can only be explained if these fish either use a style of swimming where $\frac{1}{2}\lambda_b = L$, or if they have muscle with much shorter contraction time than usual. To establish the truth there is an urgent need to measure the muscle contraction times, particularly of those species and sizes for which claims to high speed have been made, and it is important that future recordings of high speed swimming should aim to include among other evidence a tail beat frequency record.

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Evolution of the orang-utan

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The common assumption that the lineage leading to modern orang-utans has always been arboreal is based on general principles of irreversibility and parsimony rather than on any direct evidence of postcranial morphology in ancestral species. We argue here that the available evidence, albeit indirect and circumstantial, justifies the concept of a Pliocene orang-utan ancestor at least as terrestrial as the modern chimpanzee.

The fossil record of undoubted orang-utans consists solely of Pleistocene age teeth, found in sites widespread throughout South-East Asia, including the Chinese provinces of Yunnan, Kwangsi, Kwangtung and Kweichow^{1–3} and North Viet Nam (Simonetta, cited in ref. 4). The association of orang specimens at these localities with a Middle Pleistocene *Stegodon-Ailuropoda* fauna contributes little to an understanding of orang-utan evolution, because the species comprising such faunas give evidence of tropical, subtropical and temperate adaptations^{5–8}. If further study of specimens held in mainland China becomes possible, a preliminary report of fossil orang-utans at a site in Shantung Province⁹ could prove significant. This locality is more than 10° in latitude further north than the well-verified south-eastern fossil orang sites, in a region that was partial desert during maximum glacial advances and open grassland during interglacials^{10,11}.

Subfossil orang-utans, dated at 30,000–40,000 BP, have been found on Sumatra and Borneo. The maxillary occlusal surface area has been estimated to be 12.9% larger in the Sumatran subfossil, *P. pygmaeus paleosumatrensis*, than in extant *Pongo*¹².

The Chinese Middle Pleistocene subspecies, *P. p. weidenreichi*, has teeth even larger than those of the Sumatran subfossil^{12,13}. The tooth size of these fossil and subfossil species indicates that either these animals were larger bodied than the living species, with a tooth size to body size ratio that could have been similar to that of extant orangs, or that they were roughly the same body size as modern *Pongo*, but with larger teeth. Both of these conditions imply the existence of a more terrestrial species. The adult male orang-utan is already the largest arboreal animal known, and field observations suggest that larger individuals would have difficulty supporting their weight in trees. If the fossil species was larger than the modern one, adult males, at least, would have been mostly terrestrial.

If, on the other hand, they were animals with larger teeth relative to body size than living orang-utans (who have relatively large teeth among the extant great apes^{14,15}), we are aware of two plausible explanations. First, the large teeth could be a dietary adaptation. This would not be particularly significant evidence for either an arboreal or a terrestrial niche¹⁶. Alternatively, the relatively large teeth could be a consequence of rapid phyletic dwarfing in body size. The Middle Pleistocene orang-utan is a mainland species, while the subfossils and extant species are restricted to islands. It is unusual for mainland and island populations of a lineage to remain the same size¹⁷, and during the Early and Middle Pleistocene a decrease in the body size of large herbivores on islands was common. When body size undergoes rapid reduction, tooth size decreases more slowly^{18,19}, resulting in relative megadonty.

Thus, subfossil orang-utans were probably arboreal (and therefore little if any larger in body size than living orang-utans) for it seems unlikely that 40,000 yr has been sufficient for the modern arboreal specialisations to evolve. Perhaps arboreality developed from semi-terrestrial, semi-arboreal populations on the islands in response to human predatory pressures²⁰, which accelerated body size reductions. The still larger-toothed, larger-bodied Middle Pleistocene mainland orang-utans would have been less arboreal, and more chimpanzee-like in their use of terrestrial habitats.

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Additional evidence for our hypothesis comes from an examination of morphology and behaviour of the living species. In social structure and craniofacial morphology, the orang-utan is an odd animal. The sex difference in the body size of orang-utans is among the greatest of all primates^{21,22}. This is usually explained on the basis of aggressive competition between adult males^{20,21,23-27}, even though sexual selection is not clearly indicated by field observations. For example, Horr²⁴ admits that "direct evidence for male competition in the form of dominance or aggressive encounters is limited" and Leutenegger and Kelley²¹ observe that their acceptance of intrasexual selection is "partly based on negative evidence for other selective factors". While allometry²⁸, bioenergetic and reproductive constraints on female size²⁹ or a maximisation of food resources by niche separation due to body size²² remain possible factors, it is also plausible that the sex difference and large body size of orang-utans are remnants ('heritage' characters) of a more terrestrial pattern³⁰ (accompanied perhaps by a different type of social structure, of which we can know nothing).

Several aspects of dental and craniofacial morphology differ from those seen in most primate arboreal herbivores. Orang-utans have high crowned, low cusped (bunodont) cheek teeth with marked occlusal wrinkling, different from those of other catarrhine primates which exhibit well-defined cusps and shearing facets^{31,32}. The enamel on the occlusal surfaces of their cheek teeth is relatively and absolutely thicker than in any other species of living primate yet examined³³. Enamel thickness is relatively thicker on the occlusal surfaces of species of *Macaca*, *Papio*, *Theropithecus* and *Cercopithecus* (*C. aethiops*) than of species of *Colobus*, *Presbytis*, *Alouatta*, *Ateles*, *Pan* or *Gorilla*³⁴. This appears to separate species with potentially more abrasive (omnivorous) diets from those with less abrasive (folivorous, frugivorous) diets. *Pongo* is the apparent exception^{33,34}.

The mandibular condyle of the orang-utan is high above the occlusal plane and the ramus forms a relatively acute angle with the mandibular corpus. The dental arches are relatively forward in position and tilted up³⁵. Although Biegert³⁵ suggests that these changes parallel those seen in *Alouatta* and can be explained by hyolaryngeal specialisations, Hershkovitz³⁶ argues that *Alouatta* craniofacial morphology has nothing to do with hyoid hypertrophy, but rather with masticatory adaptations to herbivorous browsing, and Zingesser³⁷ confirms that several of these features are characteristic of folivory in New World monkeys.

Living orang-utans therefore have unusual (for an arboreal frugivore) dental and craniofacial adaptations. This suggests either that we do not yet have a clear understanding of the relationships between diet and anatomy in living arboreal or frugivorous primates (which is a distinct possibility), or that ancestral orangs were not arboreal frugivores. What they were we cannot say, but that they have retained a number of characteristics from ancestors adapted to doing very different things seems a real possibility.

Consideration of a terrestrial phase in orang-utan evolution goes hand-in-hand with a growing awareness within the past 5 yr that Miocene hominoid evolution is much more complex than envisioned 15 yr ago³⁸. Typical Neogene hominoids (including our hypothetical Pliocene orang-utan) were probably neither forest nor savannah dwellers, but woodland creatures. Most of these species did not resemble living hominoids. Whether some of them are ancestral to living hominoids, or whether they all became extinct without issue, it seems to us that an even wider range of possible evolutionary scenarios must be considered by students of hominoid evolution than was the case a few years ago. Selecting the correct one is probably not possible given the still poor state of the hominoid fossil record. It will not be made easier unless we realise that past hominoid morphologies, distribution and habitats were probably rather different from those of today; that we should not cling too tenaciously to principles of irreversibility or parsimony; that parallelism in hominoid evolution may have been much more widespread than some of

us have thought; and that we should intentionally begin searching for more heterodox explanations.

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Dispersal and the sex ratio

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It has been shown by Fisher that a 1:1 sex ratio should be evolutionarily stable as there would otherwise be a frequency-dependent advantage to the rarer sex¹. Hamilton pointed out that Fisher's argument depends on the assumption of population-wide random mating, and showed that a female-biased sex ratio was expected in a model in which mating occurred within small local subgroups before population-wide dispersal of mated females. We consider here the sex ratio under some other models of dispersal in a geographically structured population.

For simplicity we consider one-dimensional models, and we suppose that the habitat consists of $2M+1$ discrete patches placed round a circle and labelled $-M, \dots, -1, 0, 1, \dots, M$. In our first model we suppose that there is one mated pair in each patch, which produces k offspring and then dies. Male and female offspring disperse a distance j with probabilities p_j and q_j , respectively ($-M \leq j \leq M$). After dispersal, one pair establishes itself in each patch in the next generation, the male and female partners being chosen at random from the individuals in the patch.

To find the sex ratio which is evolutionarily stable, suppose that the pair in patch 0 has sex ratio s (producing sk sons and $(1-s)k$ daughters), while all other pairs have sex ratio r . To see whether the pair in patch 0 is at a selective advantage to other pairs, let the genes of this pair at a typical autosomal locus be coloured red. We shall now calculate then expected number of red genes in mated pairs in the next generation.

After dispersal there are $r(1-p_i)k$ males with no red genes and $sp_i k$ with two red genes in patch j , with corresponding expressions for females. The expected number of red genes in the successful pair in patch j is

$$\alpha_j = \frac{2sp_i}{sp_i + r(1-p_i)} + \frac{2(1-s)q_j}{(1-s)q_j + (1-r)(1-q_j)} \quad (1)$$

The total expected number of red genes in successful pairs in the next generation is $\alpha = \sum \alpha_j$. Writing $\varepsilon = (s-r)$ and then expanding α in a Taylor series we find that

$$\alpha = 4 + \varepsilon \left\{ \frac{2}{r} (1 - \sum p_i^2) - \frac{2}{(1-r)} (1 - \sum q_i^2) \right\} \quad (2)$$

to order ε . The evolutionarily stable sex ratio is the value of r which makes the coefficient of ε zero, given by

$$\frac{(1-r)}{r} = \frac{1 - \sum q_i^2}{1 - \sum p_i^2} \quad (3)$$

[The above argument is only approximate as it does not track the frequency of a gene determining sex ratio from one generation to the next and since we have only sought the value of r which is at a selective advantage over any mutant sex ratio s present in a single patch. We believe that the result is qualitatively correct, but a more exact analytical treatment of this problem would be valuable.]

Equation (3) can be interpreted as meaning that the evolutionarily stable sex ratio is biased in favour of the sex which disperses more widely and/or more evenly. The mechanism underlying this effect is the competition between siblings of the same sex which has been built into the model; siblings of the sex which disperses further (and/or more evenly) are less likely to be in the same patch after dispersal than siblings of the other sex. The equilibrium sex ratio in equation (3) is the point at which the advantage of producing offspring of the sex with less sibling competition is balanced by the disadvantage of producing offspring of the commoner sex. This mechanism also accounts for the female-biased sex ratio in Hamilton's model^{2,3}, as in this case there is competition between brothers for mates but no competition between sisters.

The above model assumes that mating occurs after dispersal (in contrast with Hamilton's model) and that individuals disperse independently of each other. The second assumption is more likely to be satisfied in animals dispersed passively by external physical forces (for example, marine plankton) than in animals which can control their own movement and can therefore mitigate the effect of competition for space by spacing themselves out. The assumption of independent, passive dispersal is also likely to be appropriate for plants; we shall now extend the model to the problem of resource allocation to male and female functions in hermaphrodite annual plants.

As before we suppose that the habitat consists of discrete patches round a circle. One plant grows in each patch, and each plant produces both pollen and seed. Pollen disperses a distance j with probability p_j , the seed in any patch is fertilised at random by the pollen arriving there, and then disperses a distance j with probability q_j . Of the seed arriving in a patch, exactly one is successful in establishing itself as a mature plant in the next generation.

We suppose that a plant can produce either N pollen grains or n ovules or any linear combination of rN pollen grains and $(1-r)n$ ovules ($0 \leq r \leq 1$). The parameter r is the proportion of its resources allocated to male as opposed to female functions, and is the analogue of the sex ratio in dioecious organisms. To find the evolutionarily stable value of r , suppose that a plant in a single patch has a sex ratio s while all other plants have a sex ratio r . Using the argument invoked above, we find that the evolutionarily stable sex ratio is given by

$$\frac{1-r}{r} = \frac{1+p_0 - \sum q_i^2 - \sum p_i q_{i-1} q_i}{1 - \sum p_i^2} \quad (4)$$

This is a rather complicated expression, but it is clearly possible to obtain either a male-biased or a female-biased sex ratio, depending on the relative dispersal distance of seed and pollen. In general, we may suppose that pollen dispersal has a mode at zero, so that $p_0 \geq p_i$. In this case

$$\sum p_i q_{i-1} q_i \leq p_0 \sum q_{i-1} q_i = p_0$$

so that

$$\frac{1-r}{r} \geq \frac{1 - \sum q_i^2}{1 - \sum p_i^2} \quad (5)$$

Comparing equations (3) and (5), we conclude that there is a tendency for the sex ratio to be biased towards male or female accordingly as pollen or seed disperses more, but that in addition there is some bias towards the female (seed production). This can be attributed to the fact that male (pollen) dispersal occurs in the gamete stage before fertilisation, while female (seed) dispersal occurs in the zygote stage after fertilisation.

The above argument places no restriction on selfing. If selfing is avoided, then effective pollen must come from a different plant. The argument goes through as before if we consider only effective pollen, and equation (4) remains valid if we replace p_i by the truncated distribution p'_i , defined by

$$p'_0 = 0 \\ p'_i = p_i / (1 - p_0), \quad i \neq 0$$

In general, the sex ratio will be slightly more male-biased with avoidance of selfing than if selfing is allowed.

We conclude that differential sibling competition is an important factor in determining the equilibrium sex ratio in a geographically structured population, and can lead to a bias in favour either of males or of females. It has been suggested⁴ that sibling mating is a factor which determines departures from a 1:1 sex ratio in this situation, but in our view⁵ sibling mating is only important as an indicator of the competition between brothers for mates. It is not possible to infer a general relationship between the amount of sibling mating or inbreeding and the sex ratio without considering the means by which inbreeding is caused.

Empirical evidence of the effect of sibling competition on the sex ratio has been found in the bushbaby *Galago crassicaudatus*⁶. There seems to be a male-biased sex ratio, which can be attributed to competition between female kin (sisters, and mothers and daughters) for local limiting resources of high quality food required by pregnant and nursing females.

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Measurement of gene flow in *Lupinus texensis*

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Gene flow, the movement or dispersal of genes within or among populations, is a crucial factor in determining the structure and cohesiveness of species and populations. Spatial restriction of gene flow can lead to non-random mating and to subdivision of a population into genetic neighbourhoods. On the other hand, extensive gene flow over large geographical areas can overshadow the influence of selection, leading to genetic similarity among populations and increased uniformity within a species. The extent and magnitude of actual gene flow are thus relevant to a broad range of disciplines. While the central role of gene flow is widely recognised, actual measurements of gene flow in either plants or animals are few. In plants gene flow can occur via seed or pollen dispersal. Levels of gene flow due to pollen dispersal have usually been inferred, either by marking pollen with a chemical or radioactive label for wind pollinated species or by determining the distribution of pollinator foraging flight distances for insect-pollinated species. Measurements of gene flow via pollen movement in plants have been limited to studies of a few agricultural species, where the minimum separation distance required to isolate stocks genetically was of interest^{1,2}. Here I report the measurement of the pollen component of gene flow in the insect-pollinated species, *Lupinus texensis*, compare the actual gene flow distribution with the distribution inferred from pollinator flight movements, and finally, determine the genetic neighbourhood size of this species from the pollen and seed dispersal distributions. Actual gene flow via pollen is found to be greater than would be inferred from pollinator movement alone. Gene flow and neighbourhood size are nevertheless very restricted.

Lupinus texensis Hook. (Leguminosae), the Texas bluebonnet, is a winter annual plant of widespread occurrence in central Texas. It is obligately outcrossing, and the pollen vectors are bees, predominantly *Bombus pennsylvanicus* and *Apis mellifera*. Seeds of *L. texensis* are dispersed over distances up to 4 m by the explosive dehiscence of the seed pod.

The pollen component of gene flow was studied in an experimental population of *L. texensis* using as genetic markers allozymes of the phosphoglucose isomerase-1 locus (*Pgi-1*). The *Pgi-1* locus is variable in natural *L. texensis* populations which often contain as many as five mendelian alleles. Flowering plants grown in a greenhouse from field-collected seeds were assayed

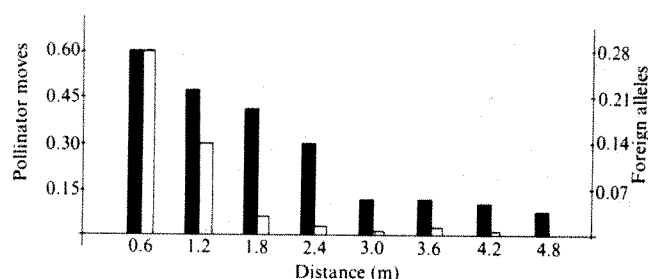


Fig. 1 Gene flow and pollinator foraging distances in *Lupinus texensis*. Gene flow (solid bars) is plotted as the frequency of foreign alleles (either *Pgi-1^f* or *Pgi-1^s*) detected in the F_1 progeny of plants located at a given distance. Pollinator moves (open bars) are plotted as the frequency of the total pollinator moves to a given distance.

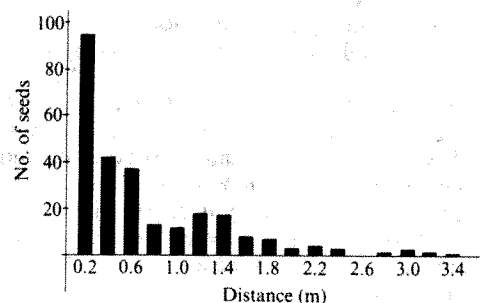


Fig. 2 Seed dispersal in *Lupinus texensis*. Seed dispersal is plotted as the number of seeds dispersed to a given distance.

for *Pgi-1* genotype and used to construct an experimental population. The experimental population comprised 91 plants spaced uniformly 0.6 m apart in a hexagonal array. Based on the behaviour of pollinators in natural populations, this array was chosen to be large enough for bees to forage without constraint. The centre of the population consisted of a core of seven plants homozygous for an electrophoretically rapidly migrating allele, *Pgi-1^f*. Along one margin of the population were plants homozygous for a slowly migrating allele, *Pgi-1^s*, while the body of the population consisted of plants homozygous for an electrophoretically intermediate allele, *Pgi-1^m*. Bees were allowed to forage freely on the plants in the experimental population for 3 days. During this time the distribution of pollinator flight distances between plants was recorded as a base for an inferred estimation of pollen gene flow. After the experimental period plants were returned to the greenhouse and the resulting fruits were marked. Of the F_1 progeny that resulted from pollinations during the experimental period 746 were assayed for *Pgi-1* genotype. Actual pollen gene flow was measured by detecting heterozygous F_1 progeny, *Pgi-1^{m/f}* if gene flow occurred from the centre of the population, or *Pgi-1^{m/s}* if gene flow occurred from the population margin.

Figure 1 presents the combined gene flow curves and the pollinator foraging flight distribution for the experimental population. Most of the pollinator moves and most gene flow are over very short distances. The actual movement of genes in the population is greater than would be inferred from the pollinator flight distribution alone. Mean gene movement, 1.82 ± 0.10 m, is almost twice as far as the mean pollinator flight distance, 0.97 ± 0.08 m. There were no significant differences between gene flow from the margin and from the centre of the population ($\chi^2 = 8.62$, $P > 0.05$). The shapes of both the pollinator flight distribution and the gene flow curve are leptokurtic, $g_2 = 11.7$ and 3.0 , respectively ($P < 0.05$). The curves differ significantly in relative proportions at each distance ($\chi^2 = 427.3$, $P < 0.05$). The disparity between the two curves is a consequence of pollen carryover from one plant to the next. Pollen carryover occurs when not all of the pollen a bee deposits on a stigma is from the flower just visited; some fraction comes from flowers visited before the most recent. Despite pollen carryover by pollinators, gene dispersal via pollen is still restricted, most gene movement being to neighbouring plants.

As gene movements by both pollen and seed are determinants of genetic neighbourhood size, the seed dispersal distribution was determined for *L. texensis*. Plants whose fruits were ripe and ready to dehisce were placed in an unobstructed area, and the actual distance of seed dispersal from parent plants was measured for 263 seeds (Fig. 2). The seed dispersal distribution is leptokurtic ($g_2 = 5.5$, $P < 0.05$), and the mean seed dispersal distance is 0.58 ± 0.04 m. Seed dispersal as well as pollen dispersal is also restricted; most seeds move but a slight distance from the parent.

Restricted gene flow within a population, such as that observed here, results in non-random mating within the population as a whole. Wright has defined the neighbourhood as that subunit of a population within which there is random mating

(panmixia)³. As there is a significant difference between the estimated gene flow from pollinator foraging distances and actual gene flow, it is of interest to know how such differences influence neighbourhood size. Neighbourhood size, N_e , is defined as the number of individuals which compose a panmictic unit, and its value is estimated by:

$$N_e = 3.6D \left(\frac{\sum p^2}{2N_p} + \frac{\sum s^2}{N_s} \right)$$

where p is the dispersal distance of genes via pollen, s is the axial seed dispersal distance, N_p is the number of pollen dispersed genes, N_s the number of seeds and D is the flowering plant density⁴. Neighbourhood size estimates are generally derived most reliably from data of experimental populations. These estimates have meaning in natural populations as well, as the nature of gene flow is determined predominately by inherent characteristics of the species, such as its modes of pollination and of seed dispersal. Except at the extremes of density, gene flow is influenced only minimally by the distribution of individuals peculiar to a given site.

In the experimental population of *L. texensis*, neighbourhood size, based on gene flow estimated from pollinator movement, is 42.2 plants and neighbourhood area is 2.8 m². The neighbourhood size based on actual gene flow is more than twice as large, 95.4 plants, and the area is 6.3 m². Pollen carryover thus has a significant effect on actual neighbourhood size.

The distribution of pollinator flight distances provides only a minimal estimate of the pollen component of gene flow in *Lupinus texensis* and most likely, in other plant species as well. Actual gene flow distance somewhat exceeds that predicted from pollinator flight distribution alone. However, gene flow due to pollen movement is still very restricted spatially. In this study, no gene migration from one margin of the population to the other was detected. The restriction of gene flow is manifest in the small estimates of neighbourhood size and area. A large body of theoretical work predicts that such limited gene flow will greatly influence the genetic structure of populations^{5,6}. As natural populations of *L. texensis* are typically orders of magnitude greater in size than the estimated neighbourhood size—often larger than 10,000 individuals and covering several hectares—they are likely to be subdivided into many neighbourhoods. Such populations are not panmictic, and should exhibit such properties as significant genetic heterogeneity, isolation by distance, and possibly inbreeding.

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Serotyping *Plasmodium falciparum* malaria with S-antigens

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The heat-stable proteins known as S-antigens which are associated with *Plasmodium falciparum* malarial infections in man show considerable serological diversity¹. Usually, different S-antigens appear in separate malarial episodes in individuals exposed to reinfection². The antigens have a worldwide distribution in endemic areas but the restricted number and characteristic specificity of S-antigens recovered from experimentally infected *Aotus* monkeys suggest that these antigens might provide suitable markers for serotyping isolates of *P. falciparum*³. Two kinds of evidence to support this idea are presented here. First, S-antigens which characterise an isolate of parasites are shown to retain their specificity over long periods of passage either *in vivo* or *in vitro*. Second, predictable mixtures of S-antigens can be recovered from cultures of *P. falciparum* after deliberately mixing isolates which each give rise to characteristic S-antigens.

The human isolates of *P. falciparum* described in this work were obtained by sampling at random acutely infected children attending the outpatients clinic at the MRC Laboratories in The Gambia, West Africa. Plasma from each blood sample was stored frozen until tested for S-antigen content by gel diffusion. S-antigens were identified by their stability to heating at 100 °C for 5 min⁴. Washed infected erythrocytes from each patient were cryopreserved in glycerol buffer at –196 °C and subsequently thawed and reconstituted by a simplified method⁵. The thawed infected erythrocytes were cultivated *in vitro* with human red cells of blood group A or O (ref. 6). The medium consisted of RPMI 1640 with 15% serum and was changed twice daily. The gas phase was 5% O₂, 7% CO₂, and 88% N₂. Pools of waste culture fluid were centrifuged at 950g to remove cellular debris and were concentrated 10-fold by negative pressure dialysis. Plasma samples were also collected from *Aotus*

monkeys infected with various named isolates of *P. falciparum*, through the courtesy of Dr W. Collins, C.D.C. Atlanta, Georgia. In other experiments, an isolate of *P. falciparum* was serially passaged through splenectomised squirrel monkeys (*Saimiri* sp.), in collaboration with Dr M. Hommel, Institut Pasteur, Paris.

The long-term stability of S-antigen expression was tested using the Ugandan Palo Alto line of *P. falciparum* which has two main S-antigens (Table 1). After more than 10 passages through *Aotus* monkeys, this line of parasites was cultivated *in vitro* in human erythrocytes for 1 month. The parasites were then passed serially through two previously infected (partially immune) *Aotus* monkeys which developed low-grade parasitaemias. The parasites which emerged were transferred by direct blood passage to splenectomised squirrel monkeys and underwent 29 serial transfers before propagation in cultures of human red cells for a further month. Finally the parasitised erythrocytes were inoculated into a squirrel monkey to produce an acute infection. The S-antigens in the plasma of this animal as well as from several of the previous squirrel and *Aotus* monkeys and also from concentrated culture fluids were compared serologically. No alteration was detected in the S-antigen specificities throughout these manipulations. Although selection of parasites

Table 1 Distribution of S-antigens in *Aotus*-adapted isolates of *P. falciparum*

Isolate	S-antigen specificities*						
	a	b	c	d	e	f	g
Malayan Camp	+	+	–	–	–	–	–
Ugandan Palo Alto	+	+	–	–	–	–	–
Salvador (St Lucia)	+	+	–	–	–	–	–
West African I	+	+	–	–	–	–	–
West African (Lagos)	–	–	+	+	(+)	–	–
Nigerian I	–	–	–	(+)	+	–	–
Haitian III	–	–	–	–	(+)	+	–
Haitian I	–	–	–	–	–	–	?†
Cambodian I	–	–	–	–	–	–	?†

* Specificities a and b correspond to S-antigens 7 and 8 in Fig. 3 in ref. 1.

† (), Trace only.

‡ Antiserum not available.

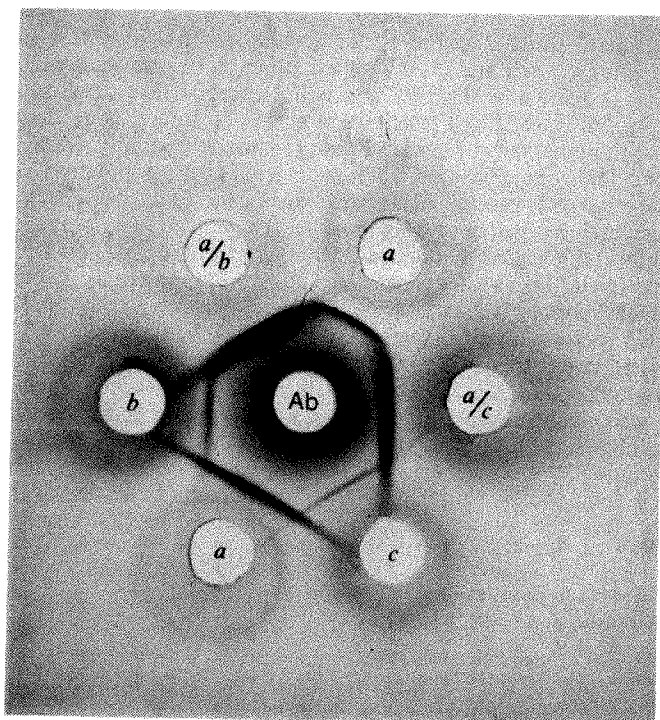


Fig. 1 Production of predicted S-antigens by three lines of *P. falciparum* cultivated *in vitro*. *a*, Plasma from *Aotus* monkey infected with Palo Alto line. *b*, *c*, Plasmas from two different human infections. *a/b* S-antigens from a mixed culture of *a* and *b* parasites. *a/c* S-antigens from a mixed culture of *a* and *c*, with *c* in a minority. *Ab*, Antiserum from an immune Gambian adult.

might have occurred at several points during the series of passages, it appeared that some of the original parasite type persisted. No new type was found despite screening with several heterospecific antisera known to react with a wide variety of S-antigens.

It is necessary to point out here that different S-antigens can be demonstrated in different lines of *P. falciparum* adapted to the *Aotus* monkey (Table 1). However, the frequency of antigen specificities 7 and 8, previously demonstrated in the Malayan Camp strain⁷, is unexpectedly high—four of nine *Aotus*-adapted isolates examined (44%). The same antigens occurred in only 16% of 50 malarious children tested at random. More surveys on these lines are desirable because recent immunological tests^{8,9} with *Aotus*-adapted lines of *P. falciparum* have been made on the assumption that lines with different names are serologically distinct.

It is not established clearly yet whether S-antigens are a direct product of the parasite genome or a host component modified in some unspecified way inside the parasitised erythrocyte. To demonstrate that different lines of *P. falciparum* express their characteristic S-antigens when grown in erythrocytes from a single source still would not indicate their parasite origin unambiguously. However, such an experiment would strongly support the hypothesis drawn from epidemiological and other studies that different S-antigens are characteristic of different isolates of parasites.

To carry out this experiment, plasma samples from 30 acutely infected Gambian children were screened to find appropriately reactive S-antigens and to select those with different specificities. Cryopreserved infected erythrocytes from the selected isolates were reconstituted and cultivated *in vitro*. Examination of concentrated culture fluids from the different lines confirmed that the expected S-antigens were present. Two of these recently derived human lines of *P. falciparum* were then mixed individually with the Palo Alto line to produce mixed cultures. Gel diffusion analysis of concentrated culture fluids

from each mixed culture showed that they now contained the predicted mixture of S-antigens (Fig. 1).

These studies suggest that S-antigens can be used as stable markers of isolates of *P. falciparum* grown either in experimental hosts or in cultures. The findings support our earlier hypothesis¹ that S-antigens reflect considerable diversity in natural populations of *P. falciparum* and that mixed infections with different types occur commonly. This last point has also been confirmed by the demonstration of mixed isozymes of parasite glycolytic enzymes in extracts of infected erythrocytes¹⁰. S-antigens, however, provide a much more extensive marker system than the isozyme and drug resistance differences described by others. Immunological experiments on selective destruction of parasites producing different S-antigens in mixed cultures might throw light on the question of strain specific immunity to *P. falciparum* for which there is evidence in experimental infections of man¹¹ and the *Aotus* monkey^{8,12}.

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Mouse C_{μ} heavy chain immunoglobulin gene segment contains three intervening sequences separating domains

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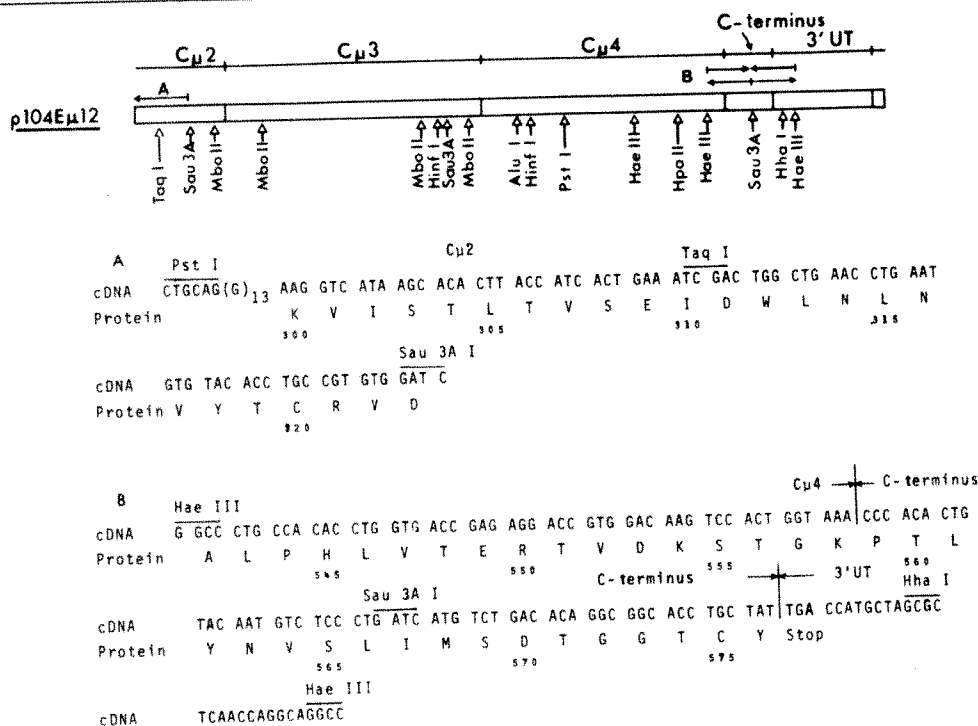
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The IgM molecule is composed of subunits made up of two light chain and two heavy chain (μ) polypeptides. The μ chain is encoded by several gene segments—variable (V), joining (J) and constant (C_{μ})^{1,2}. The C_{μ} gene segment is of particular interest for several reasons. First, the μ chain must exist in two very different environments—as an integral membrane protein in receptor IgM molecules (μ_m) and as soluble serum protein in IgM molecules into the blood (μ_s). Second, the C_{μ} region in μ_s is composed of four homology units or domains ($C_{\mu}1$, $C_{\mu}2$, $C_{\mu}3$ and $C_{\mu}4$) of approximately 110 amino acid residues plus a C-terminal tail of 19 residues^{3,4}. We asked two questions concerning the organisation of the C_{μ} gene segment. (1) Are the homology units separated by intervening DNA sequences as has been reported for α (ref. 5), γ_1 (ref. 6) and γ_{2b} (ref. 7) heavy chain genes? (2) Is the C-terminal tail separated from the $C_{\mu}4$ domain by an intervening DNA sequence? If so, DNA rearrangements or RNA splicing could generate hydrophilic and hydrophobic C-terminal tails for the μ_s and μ_m polypeptides, respectively. We demonstrate here that intervening DNA sequences separate each of the four coding regions for C_{μ} domains, and that the coding regions for the $C_{\mu}4$ domain and the C-terminal tail are directly contiguous.

Fig. 1 Restriction map and sequences of p104E μ 12. The double-stranded cDNA segment was originally inserted into the *Pst* site of pBR322 by a dC-dG tailing procedure which reconstituted the *Pst* sites at each side¹⁶ (W. Rowenkamp and R. Firtel, personal communication). Mapping was initially done by multiple digests of the whole plasmid. To locate *Mbo*II sites and to confirm some *Sau*3A1 and *Hae*III sites, isolated restriction fragments were 5' end-labelled, cut to separate the labelled ends, and partially digested with the appropriate restriction enzymes. DNA sequences (in regions indicated by arrows) permitted the map to be aligned with the C μ amino-acid sequence⁴.

All the restriction sites mapped are consistent with the coding sequence. There are no sites in the insert for *Eco*RI, *Bam*HI, *Kpn*I or *Xba*I. A and B show DNA sequences from p104E μ 12. Sequences were obtained by the method of Maxam and Gilbert¹⁷, with minor modifications using the 5' end-labelled restriction fragments indicated in the restriction map. Sequence A was determined in one direction only; nucleotides printed in lower case type were ambiguous and have been supplied from the coding requirements. Sequences B was determined in both directions and was unambiguous.



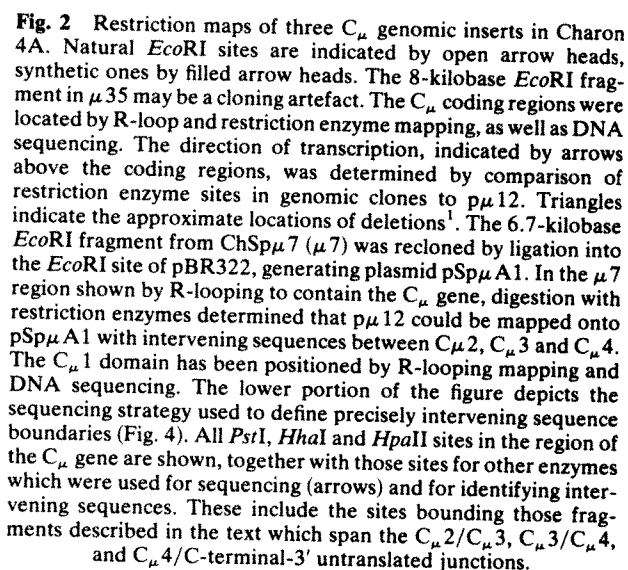
A recombinant plasmid, p104E μ 12 (p μ 12), containing a cDNA sequence from the heavy chain mRNA of the IgM-producing myeloma tumour, M104E, was constructed and characterised by restriction mapping and partial DNA sequence analysis (Fig. 1). A comparison of these DNA sequences with the protein sequences of the M104E myeloma μ chain⁴ indicates that p μ 12 contains C μ coding sequences extending from residue 300 to the C-terminus of the μ chain at position 576. The codon for the C-terminal tyrosine is followed immediately by a stop codon (UGA).

The p μ 12 probe was used to screen several genomic libraries constructed in the vector Charon 4A—a partial *Eco*RI library from the DNA of IgA-producing myeloma M603 (refs 5, 8), a partial *Eco*RI germ-line library from mouse sperm DNA¹ and a partial *Hae*III+*Alu*I germ-line library from sperm DNA¹. Southern blot analyses of *Eco*RI-digested mouse sperm and M603 DNA using the p μ 12 probe showed identical 12.2-kilobase C μ bands¹. This suggests that both the myeloma and germ-line libraries contain the C μ gene segment in the germ-line or unrearranged state. Figure 2 shows the restriction enzyme patterns of three genomic clones. ChSp μ 27 (μ 27) from the sperm library and Ch603 μ 35 (μ 35) from the M603 library contain *Eco*RI restriction fragments of 10.2 and 9.8 kilobases, respectively, which hybridise to the p μ 12 probe. The μ 27 and μ 35 *Eco*RI fragments are slightly smaller than the 12.2-kilobase band observed in *Eco*RI-digested sperm and M603 DNAs. We believe that this discrepancy is caused by deletions in genomic DNA flanking the C μ gene segment which occurred during growth or amplification of the recombinant phages¹. Preliminary Southern blot comparisons of germ-line and μ 27 DNAs localise these deletion(s) to within 1 kilobase 5' to the C μ coding region (M. D., unpublished results) (Fig. 2). A similar result has been obtained by others with C μ -containing clones isolated from a mouse liver DNA library (F. Blattner and N. Newell, personal communication). However, as we show below, the C μ coding sequences of each clone are identical within the limits of our analyses and presumably represent the true germ-line arrangement of the C μ gene segment.

R-loop analyses using heavy chain mRNA from myeloma tumour M104E were performed and similar results were

obtained for all three genomic clones. Representative electron micrographs are shown in Fig. 3. A total of 90 R-loop molecules were analysed, 62 from μ 35 DNA and 28 from μ 27 DNA. A small number of the molecules (6%) showed four single-stranded DNA loops of approximately equal length which we interpret to be four C μ coding regions separated by small, base-paired intervening sequences (Fig. 3a, b). A larger fraction (74%) showed two or three loops, indicating that one or two of the intervening sequences were base-paired while the others remained single-stranded. Often a small bulge appeared at a reproducible point in the double-stranded DNA-RNA hybrid, indicating the position of the single-stranded intervening sequence (Fig. 3d). The remaining 20% of the R-loop molecules showed one large loop. Most of these had small bulges in the double-stranded portion at the positions of one or more single-stranded intervening sequences. The total length of the four coding regions was 1.6 ± 0.2 kilobases and the average size of the loops was 375 ± 51 , 364 ± 58 , 361 ± 60 and 369 ± 73 base pairs. The size of the three intervening DNA sequences could not be accurately determined by this procedure. In addition, R-loop measurements located the C μ coding region $\sim 3.6 \pm 0.36$ kilobases from the 3' end of the 9.8-kilobase *Eco*RI fragment of μ 35. The R-loop structures that we observe indicate that the C μ gene contains three intervening sequences which seem to separate regions coding for the four structural domains of the μ heavy chain.

The coding regions were located more precisely by restriction mapping (Fig. 2). We mapped *Hinf*I, and *Sau*3A1 sites in the region of the four C μ domains in order to measure the sizes of the intervening sequences. A *Sau*3A1 fragment spanning the C μ 2–C μ 3 junction is 331 base pairs long in the p μ 12 cDNA clone and 620 base pairs long in the germ-line clone ChSp μ 7 (μ 7). Therefore, an intervening DNA sequence of 289 ± 15 base pairs exists between C μ 2 and C μ 3. A *Hinf*I fragment spanning the C μ 3–C μ 4 junction is 122 base pairs long in the cDNA clone and 230 base pairs long in the germ-line clone, while a *Sau*3A1–*Pst*I fragment is 147 and 254 base pairs long in the two clones, respectively. Accordingly, there are 108 ± 10 base pairs of intervening sequence between C μ 3 and C μ 4. The C μ 1–C μ 2 junction was not available in a cDNA clone, but the entire



All of the boundaries of the coding and intervening sequences in the C_μ gene segment were sequenced using the strategy shown in Fig. 2. Figure 4 gives the sequences of the splice sites and shows that there is terminal redundancy about each of the intervening sequences. At the downstream splice sites between J_H and $C_\mu 1$, and between $C_\mu 1$ and $C_\mu 2$, the noncoding sequence is identical to the preceding coding sequence for 7 and 8 nucleotides, respectively, before the indicated splice points. The precise splice points can be designated according to the $\text{the/GT} \cdots \text{AG/rule}^9$, and the junction sequences are then found to conform generally to the 'consensus' RNA splicing sites (Fig 2)⁹⁻¹². The intervening sequences occur in codons 127, 230, 340 and 446. These are identical to the C_μ domain boundaries as far as they could be determined from protein sequence homologies⁴.

Restriction mapping also indicates that the C_μ 4 coding region and the C-terminal tail are not separated by an intervening sequence but are continuously encoded in the germ-line DNA. This junction is spanned in μ 12 by a *Pst*I-*Hha*I fragment (Fig. 1), which is 297 base pairs long according to the coding requirements. The corresponding fragment from a subclone of μ 7 (Fig. 2) co-migrates with the μ 12 fragment to an accuracy of ± 15 base pairs. This fragment was isolated from the μ 7 sub-

Our current observations on the C_{μ} genomic clones, in conjunction with previous studies on C_{α}^5 and $C_{\gamma 1}$ and $C_{\gamma 2b}$ genomic clones, suggest that all immunoglobulin C_H genes will contain intervening sequences separating the regions coding for structural domains. Although the function of intervening sequences in eukaryotic genes remains unclear, their positioning precisely at the interdomain boundaries of immunoglobulin C_H genes suggests that the positions of the intervening DNA sequences may have some role in the evolution of immunoglobulin genes. As individual immunoglobulin domains probably encode dis-

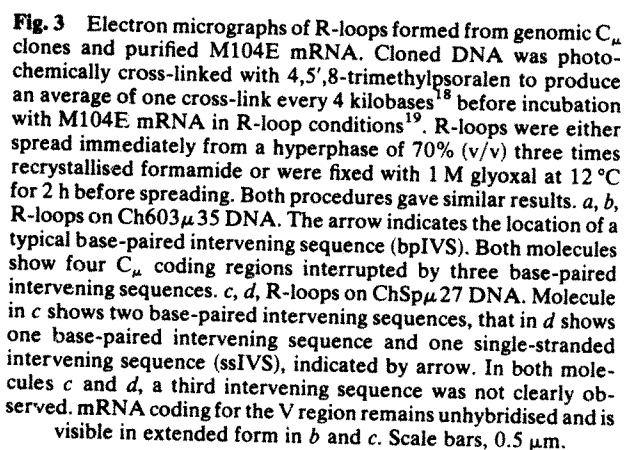


Fig. 4 Junctional sequences in the germ-line C_{μ} gene. *The location and sequence of J_{H107} in ChSp μ 27 is from ref. 2.

Junctions	Intervening sequences	
$J_{H107}^*/C_{\mu}1$	ValSerSer GTCCTCTCAGTAAGCTGGCTT---	7.5 \pm 0.8 kb -----GTCCTCAGAGATCAG GluSerGln
$C_{\mu}1/C_{\mu}2$	ProIlePro CCCATTCCAGTAAGAACCAAA---	107 bp ---ACCTTGACCTTTTCATTCCAGTGTGCGCA AlaValAla
$C_{\mu}2/C_{\mu}3$	CysAlaAla TGTGCTGCCAGTGTAGTGGCTGG---	289 \pm 15 bp ---CAGTGTCTCTTGACTGCAGGTCCCTCC SerProSer
$C_{\mu}3/C_{\mu}4$	LysProAsn AAACCCAATGTAGGTATCCCCC---	108 \pm 10 bp ---ACTACTGTCTTCATTACAGAGGTGCAC GluValHis
Consensus sites	5' AGTAAGTA-----TTTTTTTTTCTTNCAG 3'	

tinct functions, the presence of intervening DNA sequences at the domain boundaries may facilitate the rearrangement of domain coding regions and thereby generate new combinations of heavy chain domains for selection, thereby speeding up the evolution of immunoglobulin genes^{8,13}. Alternatively, it also has been proposed that intervening DNA sequences inhibit recombination and, accordingly slow the evolution of eukaryotic genes^{14,15}.

Our studies on the organisation and structure of the C_{μ} gene segment place several constraints on models for the difference between μ_m and μ_s chains. First, Southern blot analyses of embryo or germ-line DNA with the μ 12 probe show only strongly hybridising bands corresponding to a single C_{μ} gene segment which is present in the μ 35, μ 27 and μ 7 clones. This is true for digests with *Eco*RI or *Hinc*II (ref. 1), as well as for *Bam*HI or *Hha*I (M. D., unpublished results). We have isolated 10 independent genomic clones which hybridise to μ 12, and all of these seem to contain the same C_{μ} gene segment (M. D., K. C. and P. E., unpublished results). Thus, these restriction mapping and gene cloning results strongly suggest that there is only one C_{μ} gene segment in the BALB/c genome. If so, the μ_s and μ_m chains must be encoded by the same C_{μ} gene segment. Second, a DNA rearrangement during B-cell development to generate alternative $C_{\mu m}$ and $C_{\mu s}$ gene segments is unlikely. In B-cell development, the μ_m chain is expressed before the μ_s chain. Thus, a putative DNA rearrangement should alter the 3' structure of the expressed $C_{\mu s}$ gene segment. However, our results show that the 3' end of the C_{μ} gene in the μ 12 cDNA clone is identical to the 3' end of the C_{μ} gene in the μ 7 germ-line clone, thus ruling out the possibility of a DNA rearrangement at the 3' coding region of the $C_{\mu s}$ gene segment. Finally, we can rule out a simple post-translation cleavage of a larger μ_m chain to create the μ_s chain because the μ_s coding sequence is followed immediately by a stop codon (Fig. 1).

Two models to explain the origins of μ_m and μ_s still appear plausible: (1) the μ_m chain is generated from the μ_s chain by a novel type of post-translational modification; and (2) a different COOH-terminal coding region for the μ_m chain does exist. A large nuclear RNA transcript could give rise either to μ_s mRNA or, alternatively, to μ_m mRNA by RNA termination, cleavage and/or splicing. We are currently studying the μ RNAs from a B-cell lymphoma which produces only membrane IgM to test these models.

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Anomalous phenotype in thymic acute lymphoblastic leukaemia

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Malignant blast cells in the various forms of acute leukaemia seem to be 'frozen' at early stages of haematopoietic and lymphoid cell maturation. These blasts express relatively stable morphological, enzymatic and antigenic phenotypes which may largely represent the normal gene products of the corresponding precursor cells¹⁻⁴. A number of findings support these suggestions. The phenotype of the common form of acute lymphoblastic leukaemia (cALL) corresponds to the phenotype of normal small non-T, non-B cells of lymphoid morphology detected in low numbers in the normal and regenerating infant bone marrow^{4,5}. Also, the phenotype of blast cells in Thy-ALL corresponds approximately to that of cortical thymocytes or their immediate precursors⁶⁻⁹. Leukaemia-specific (virus-induced or mutant) changes or aberrant expression of normal gene products may also occur in leukaemia. To describe such phenomena, detailed single cell studies comparing leukaemic cells with their appropriate normal (frequently very rare) counterparts are needed. In this study we have performed such a comparative study and find that the human Thy-ALL blasts express unexpectedly high amounts of HLA-A, -B and -C antigens: the sensitive single cell assays used failed to find normal cells (in infant and fetal thymus, and in bone marrow) which express the exact phenotype of Thy-ALL blasts.

Both cALL and Thy-ALL blast cells express large amounts of terminal deoxynucleotidyl transferase (TdT). This nuclear enzyme can be labelled by purified rabbit antibodies to TdT in

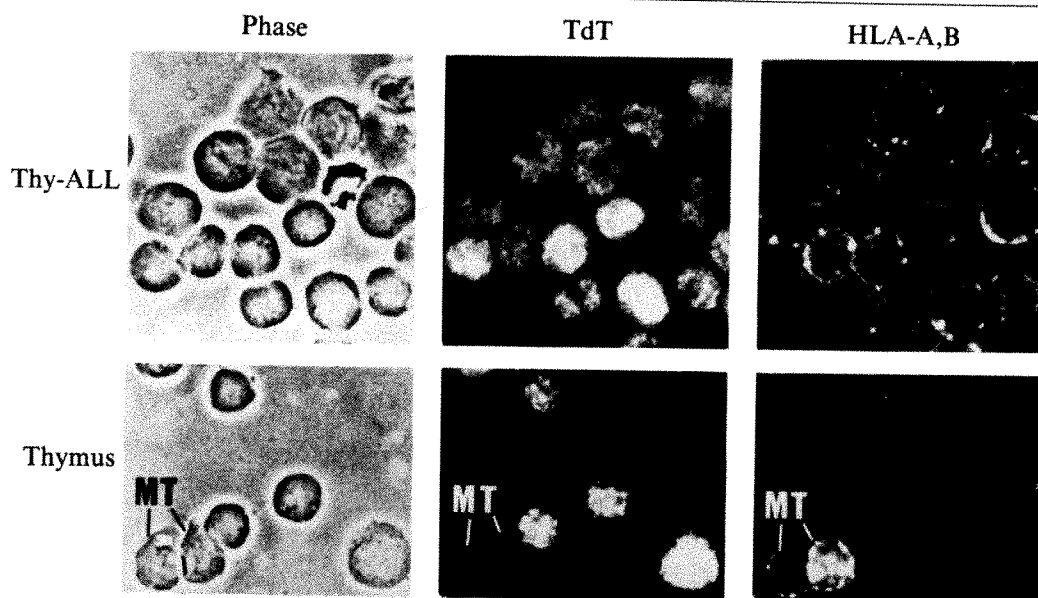


Fig. 1 Expression of HLA-A, -B and -C antigen and terminal transferase (TdT) on thymic ALL (leukaemic) blast cells and on normal thymocytes. Cells were incubated with W6/32 antibody (detecting core determinants of HLA-A, -B antigens)¹¹, washed and labelled with goat anti-mouse Ig-TRITC (red). The cells were smeared in a cytocentrifuge and restained for nuclear TdT by purified rabbit anti-TdT antibody¹⁰ followed by washing and a goat-anti-rabbit-Ig-FITC second layer (green). The preparations were photographed by filters selective for TRITC (HLA-A, -B and -C) and FITC (TdT). MT: putative medullary thymocytes. Additional studies have shown that in both Thy-ALL and thymocyte suspensions >95% of cells were strongly positive with anti-T cell serum (HuTLA⁺) and negative with anti-Ia antisera (Ia⁻)⁵.

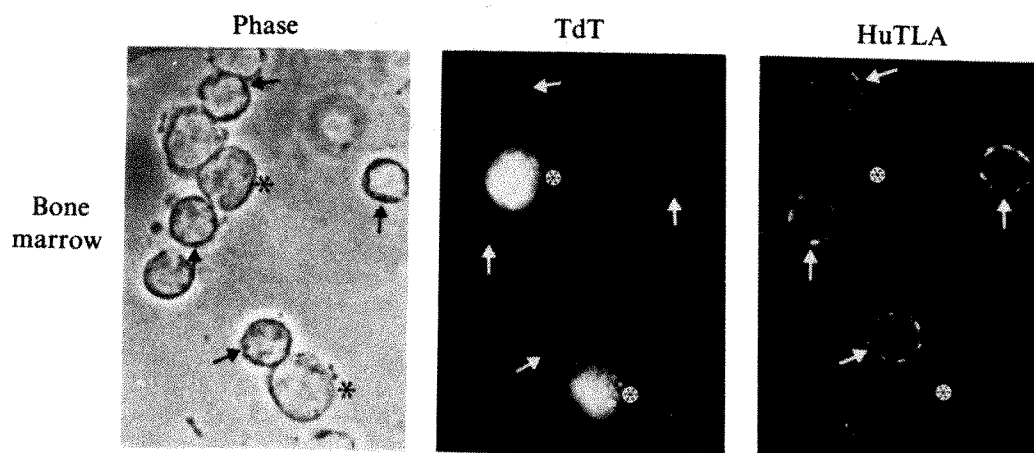


Fig. 2 In the normal human bone marrow T lymphocytes (HuTLA⁺, TdT⁻; arrows) and TdT⁺ precursor cells (HuTLA⁻, TdT⁺; asterisks) represent different populations. Cells were incubated with rabbit anti-HuTLA, labelled with goat-anti-rabbit-TRITC (red) and smeared. This was followed by restaining with rabbit anti-TdT and goat-anti-rabbit-FITC (green). Additional studies have shown that TdT⁺ bone marrow cells are HLA-A, -B and -C positive (<99%) and mostly Ia⁺ (>85%). None of these cells express HTA-1 (thymocyte-specific) antigen (see ref. 5).

indirect immunofluorescence (IF) test using fluorescein (FITC)-conjugated goat anti-rabbit immunoglobulin as second layer¹⁰. The test can conveniently be combined with other IF tests for various membrane or cytoplasmic antigens using tetra-rhodamine (TRITC)-labelled reagents (Table 1) and has been used both for the diagnosis of leukaemia and for the analysis of early lymphocyte maturation in man^{5,8}. Here, the comparative single cell assays on leukaemic and normal TdT-positive populations indicate that a large proportion of Thy-ALL blasts show anomalous phenotypic characteristics which could not be observed on normal TdT positive infant cells and fetal thymocytes.

The first relevant point was shown in suspensions of normal infant thymus labelled for nuclear TdT and core determinants of HLA-A, -B and -C antigens (using W6/32 monoclonal antibody; Fig. 1). No TdT⁺ cells had detectable HLA-A, -B and -C antigen in over 2,000 thymocytes analysed. These TdT⁺ cells

were cortical thymocytes expressing HTA-1, which is a cortical thymocyte-specific antigen (a possible homologue of mouse TL antigen¹²). Then the study was extended to the relatively rare (1-3%) TdT⁺ lymphoblasts in the infant thymus. These cells express only moderate amounts or no HTA-1 (ref. 8). When stained with Giemsa, these large blasts show a dark blue cytoplasm (putative infant pro-thymocytes). More than 400 of these TdT⁺ blasts were analysed but no HLA-A, -B, or -C positive blast cells were seen. On the other hand TdT⁻ (medullary; HTA-1⁻) thymocytes were brightly HLA-A, -B and -C positive. Thus the expression of TdT and HLA-A, -B and -C in normal thymocytes seems to be mutually exclusive for all the cells of the human thymus.

In marked contrast, in all six cases of Thy-ALL studied as well as in the Thy-ALL cell line studied (HPB-ALL; see phenotypic characteristics in Table 1) TdT⁺ blast cells expressed moderate to large amounts of HLA-A, -B and -C (Fig. 1). This was in spite

Table 1 Analysis of Thy-ALL blasts in comparison with normal infant and fetal thymocytes and TdT⁺ bone marrow cells

Markers	Normal child thymus All cells	Normal child thymus Large cells	Fetal thymus	Normal child bone marrow	1	2	Thy-ALL patients 3	4	5	6	Thy-ALL line HPB-ALL
% Of cells in the total population											
TdT ⁺	65	3	6 [‡]	4	82	92	84	98	95	35	54
E-rosette	92		85	5	55§	50	<5	NT	<1	52	37
% Of cells within the TdT ⁺ population*											
HLA-A,B,C [†]	<0.1	<0.1	<1	>99	>95	88	90	90	90	80	98
HuTLA	97	88	+	<0.1	>95	>95	>95	>95	>95	>95	98
Ia-like	0	0	-	93	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
HTA-1	88	62	+		16	32	5	0.5	0.2	<0.1	47
HLe-I	94	50	NT	34	35	4	68	2	27	<1	30

Viable cell suspensions were labelled with various antisera (see below) in indirect immunofluorescence test using tetrahydroamine (TRITC)-labelled second layers. Of the membrane-labelled cells smears were made in a cytocentrifuge and fixed for 30 min in cold methanol. These fixed smears were incubated with rabbit anti-TdT antibodies (eluted from TdT antigen column; ref. 10), washed and stained with goat anti-rabbit immunoglobulin labelled with fluorescein (FITC). The nuclear TdT staining (FITC) is easily distinguishable from the membrane staining (TRITC). The study was focused on the TdT⁺ cells in the different samples and the TdT⁻ cells were ignored. The samples studied contained different proportions of TdT⁺ cells (as shown). NT, not tested.

*Results are expressed as cells stained with membrane markers within the TdT⁺ population studied.

†The antibodies used (as first layers) to detect membrane markers were as follows: anti-HLA-A, -B and -C antibody was a mouse monoclonal reagent, W6/32, detecting a core HLA-A, -B and -C antigenic determinant, and was used as culture supernatant at 1:10 final dilution; anti-HuTLA serum was a rabbit anti-monkey thymocyte serum absorbed with human red cells, B lymphoid and myeloid leukaemic cells⁵ and used at 1:25 final dilution; a rabbit anti-Ia-like serum was made in chickens against purified human Ia-like (p28,33) antigens⁵ and was used at 1:25 dilution; anti-HTA-1 antibody was a mouse monoclonal antibody NA1/34, specifically reactive with human cortical thymocytes¹², and used as peritoneal exudate at 1:400 final dilution⁸; anti-HLe-I reagent was also a monoclonal antibody (2D1), strongly reactive with thymocytes and T and B lymphocytes, while only weakly reactive with myeloid cells. This latter antibody was used in the form of culture supernatants at 1:5 final dilution⁸. The second layers (goat-anti-mouse-Ig-TRITC, goat-anti-rabbit-Ig-TRITC and sheep-anti-chicken-TRITC) were from Nordic Labs. Both first and second layers were titrated and used at saturating condition with no detectable nonspecific staining (see details in ref. 8).

‡In the fetal thymus the few TdT⁺ cells stain extremely weakly. These cells are small HLA-A,B⁻, HTA-1⁺ thymocytes¹⁷.

§Some of these samples were tested 2 d after being obtained; the E⁺ values could therefore be artificially low.

||Weak staining.

of the fact that a variable proportion of the blast cells also expressed HTA-1.

As there was a marked discrepancy between the staining pattern of infant thymocytes and Thy-ALL blasts, we have investigated normal fetal thymocytes. The possibility that Thy-ALL might derive from fetal thymocytes has been raised by Gatten *et al.*¹³ and Stein *et al.*⁷ who showed that these cell types, unlike mature cortical thymocytes, carry receptors for activated C3 and form EAC rosettes. Nevertheless, in the three fetal thymus samples (at 14, 17 and 19 gestational weeks) simultaneously analysed for nuclear TdT and membrane HLA-A, -B and -C expression no cells expressed both markers. The vast majority of cells (90–95%, including most HTA-1⁺ cells) were TdT⁻. These results were in accord with the low or undetectable TdT values observed in the same fetal human thymus samples by the biochemical assay (G. J., K. Ganeshaguru and A. V. Hoffbrand, unpublished observation) and with observations in chicken¹⁴, mice and rats¹⁵, which also showed that fetal thymocytes are TdT⁻.

In the final part of the study we postulated that perhaps Thy-ALL blast cells express the phenotypic characteristics of rare bone marrow (BM) cells. BM samples from 5–12-year-old children with no haematological malignancy were analysed with various double markers. The phenotypes of these TdT⁺ BM cells were, again, different from that of both Thy-ALL blasts and thymocytes. Unlike thymocytes and Thy-ALL blasts, BM TdT⁺ cells failed to express HuTLA and HTA-1 (Fig. 2; see also ref. 5). All TdT⁺ BM cells (>99%) were brightly stained for HLA-A, -B, and -C; and the vast majority (>85%) were also brightly stained for Ia-like antigens (Ia⁺). This agrees with previous results⁵, suggesting that the phenotypic characteristics of TdT⁺ BM cells were similar to the phenotype of cALL blasts and different from those of Thy-ALL blasts.

In conclusion, our results indicate that human leukaemic blasts and cell lines of Thy-ALL phenotype (HuTLA⁺, E⁺, Ia⁻, HTA-1⁺) have an unexpected expression of HLA-A, -B, and -C antigens when compared to small cortical thymocytes or putative large TdT⁺ thymic prothymocytes. There are at least three possible interpretations of our findings. It is possible that even the sensitive single cell assays used are unable to detect TdT⁺,

brightly HuTLA⁺, HLA-A, -B and -C⁺, Ia⁻ bone marrow precursor cells (possibly bone marrow prothymocyte) from which Thy-ALL might develop because these cells are exceedingly rare (<10⁻⁴). It is also conceivable that the generation of HLA-A, -B and -C antigens on cortical thymocytes is associated with the malignant change (for example the rare HLA-A, -B, and -C⁺ thymic cells might be predisposed to undergo a malignant transformation) and therefore most Thy-ALL show this peculiar phenotype. It seems more likely, however, that within the intact thymic cortex microenvironmental effects modulate (that is, they actively suppress) the expression of HLA-A, -B and -C antigens on normal cortical thymocytes as part of the physiological interplay between epithelial and lymphoid elements. This possibility is in line with the experimental observations that only during this special cortical development stage do T-lineage cells lack the easily detectable HLA, -B and -C antigens^{16,17}. Thus, although Thy-ALL blasts may have originated from a thymic precursor cell (HuTLA⁺, TdT⁺, Ia⁻), they exhibit HLA-A, -B and -C antigens perhaps due to the loss of thymic microenvironmental control during the disseminated phase of the disease.

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Nonsteroidal compounds which bind epididymal androgen-binding protein but not the androgen receptor

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In the testis of most mammals the Sertoli cells of the seminiferous tubules secrete into the lumen an androgen-binding protein (ABP). This glycoprotein is of interest because it reflects Sertoli cell function and responsiveness to follicle-stimulating hormone¹. In addition, ABP binds testosterone produced by the Leydig cells and the hormone-protein complex reaches the epididymis² where it could play a role in sperm maturation³⁻⁶. ABP is distinct from the androgen receptor, an intracellular protein which binds testosterone and its hormonally active metabolite 5 α -dihydrotestosterone (DHT) in target tissues⁷. In some species, but not the rat, a testosterone-oestradiol binding globulin (TeBG) circulates in blood⁸. Although radioimmunoassays for TeBG⁹ and ABP¹⁰ have been developed, most methods for assaying these proteins and the androgen receptor rely on their ability to bind ³H-labelled androgens. As all three proteins bind testosterone and DHT, accurate assay of any one protein awaits development of a specific label. A specific ligand for ABP would also be very valuable for further study of the physiologic role of ABP. We¹¹ and others¹² have investigated earlier suggestions² that the binding sites on ABP and the androgen receptor have different steroid specificities. Steroids were found that have a higher affinity for ABP than for the receptor. We now report on dicyclohexane derivatives which bind with high affinity to the testosterone-binding site on ABP but do not interact with the androgen receptor.

The dicyclohexane derivatives shown in Table 1 were synthesised by catalytic perhydrogenation of diethylstilboestrol (III, V) or of mesohexestrol (IV). Chromic oxidation of these diols yielded the corresponding diketones (I, II). All compounds were purified and characterised as part of a project on the androgenic activity of nonsteroidal molecules¹³. We first studied their effect on the interaction of androgens with ABP from rat epididymis. Specific binding was determined by a charcoal adsorption assay which we have shown to give results identical to those obtained by the classical procedure of steady-state polyacrylamide gel electrophoresis¹¹. As illustrated in Fig. 1a, compounds I, II and III could inhibit completely the binding of DHT to ABP. This effect appeared to be specific since compounds IV and V at a 2,000-fold excess produced less than 20% inhibition. The question of whether the inhibition was competitive or not was then investigated. ABP binding of DHT was studied at increasing concentrations of the latter in the presence of fixed concentrations of the analogues. Lineweaver-Burk plots of data obtained at equilibrium confirmed that DHT interacts with a single class of sites as demonstrated earlier by Scatchard analysis¹¹ and suggested that the nonsteroidal compounds interfere with DHT binding by competitive inhibition (Fig. 1b). The equilibrium affinity constants calculated from these plots are listed in Table 1. In other experiments, 1 μ M nonradioactive DHT or compound I was added to preformed

³H-labelled DHT-ABP complex. In both cases, binding was completely reversible; the time-dependent decrease in specifically-bound radioactivity followed the same first-order kinetics (half life 5.8 min) with compound I as with DHT. Thus, the properties of the binding site for the natural androgen were not modified by the presence of compound I.

The effect of the dicyclohexane derivatives on the binding of androgens to their intracellular receptor protein was quite different. Cytosol from rat ventral prostate was incubated with ³H-labelled methyltrienolone (RU 1881), a specific ligand for the androgen receptor¹⁴. In conditions where testosterone and

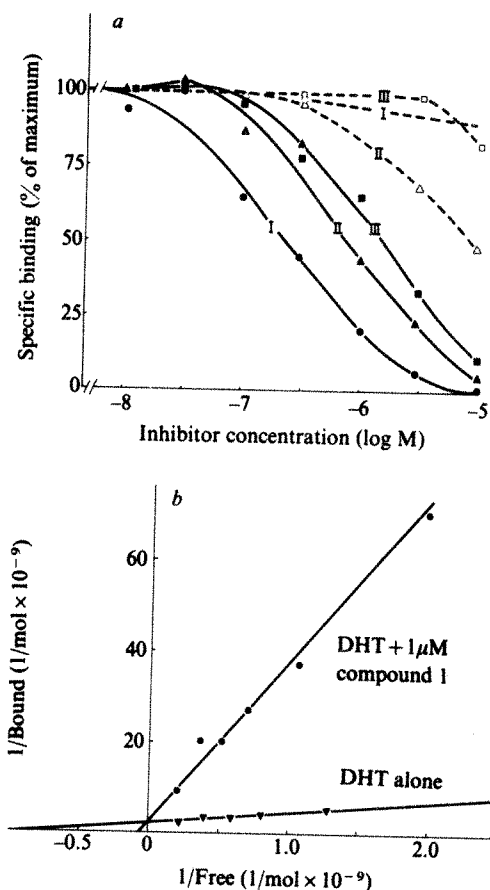


Fig. 1 Binding of dicyclohexane derivatives to ABP (closed symbols) and to the androgen receptor (open symbols). Epididymes and ventral prostates obtained from Wistar rats 24 h after castration were homogenised at 0 °C in 20 mM Tris-HCl buffer (pH 7.4) containing 1.5 mM EDTA and 10% (v/v) glycerol without (epididymis) or with (prostate) 0.25 mM dithiothreitol. Homogenates were centrifuged at 200,000g for 60 min at 0 °C to yield the cytosol. Androgen receptor in epididymis cytosol was inactivated by freezing (-20 °C) and thawing (30 °C, 30 min) in the absence of dithiothreitol, conditions to which ABP is insensitive¹¹. *a*, Epididymis cytosol was incubated for 3 h with 1.5 nM ³H-DHT in the presence of the indicated concentrations of inhibitors (see Table 1 for nomenclature) and specific binding to ABP was determined as described¹¹. Briefly, unbound hormone was removed from the 0.4 ml incubation mixtures by adding 0.2 ml of activated charcoal suspension (Norit A 50 mg ml⁻¹, dextran 5 mg ml⁻¹) and sedimenting the charcoal after 2 s of agitation. Nonspecific binding was calculated from parallel incubations containing a 500-fold excess of nonradioactive DHT. Prostate cytosol was incubated with 2.5 nM ³H-methyltrienolone for 16 h for determination of binding to the androgen receptor¹¹, in absence or presence of the inhibitors. Specific binding is expressed as a percentage of maximum binding seen in the absence of inhibitors (7,100 d.p.m. per ABP assay and 7,340 d.p.m. per receptor assay). Nonspecific binding was 10 and 27% of total binding for ABP and the receptor, respectively. *b*, Double-reciprocal plot of ABP binding at increasing ³H-DHT concentrations in the absence or presence (1 μ M) of inhibitor.

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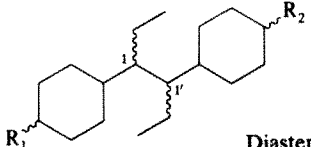
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DHT completely inhibited methyltrienolone binding, none of the compounds except II had any effect. Compound II displayed some inhibition at high concentrations (Fig. 1a). From double-reciprocal plots, this inhibition was found to be competitive.

Thus, compounds I and III specifically bind to ABP but not to the androgen receptor. Androgens are known to bind to the glucocorticoid receptor, in which system they behave as antagonists¹⁵. However, none of the nonsteroidal compounds bound to the glucocorticoid receptor from rat hepatoma cells. Unlike diethylstilboestrol from which it is derived compound I, which has the highest affinity for ABP, did not bind to the oestrogen receptor from rat uterus.

Since ABP has many characteristics in common with TeBG¹⁶, we wondered whether the nonsteroidal compounds also bound to TeBG. As this protein is not found in the rat, the test was performed on human TeBG. Human male plasma was diluted 10-fold with 20 mM Tris-HCl, 1.5 mM EDTA, 10% (v/v) glycerol, pH 7.4 and agitated for 45 min at room temperature with 1% (w/v) activated charcoal to remove endogenous steroids. After centrifugation the supernatant was incubated with 5 nM ³H-labelled DHT for 3 h without or with 10 μ M nonradioactive DHT and specific binding was determined as described for ABP. A 2,000-fold excess of compound III was required for inhibition of the binding of DHT to TeBG by 30%.

Table 1 Dicyclohexane derivatives studied

Compound			Diastereo-isomerism at C-1,1'	K_a (M ⁻¹)
	R ₁	R ₂		
I	= 0	= 0	Racemic	12.0×10^6
II	= 0	= 0	Meso	4.8×10^6
III	e OH, a H	a OH, e H	Racemic	3.5×10^6
IV	e OH, a H	e OH, a H	Meso	0.8×10^6
V	e OH, a H	e OH, a H	Racemic	0.6×10^6

e, equatorial; a, axial (see ref. 13). K_a is the equilibrium association constant (0 °C) for rat ABP calculated for double-reciprocal plots as in Fig. 1.

In these conditions, the other compounds had no effect while, as expected, oestradiol completely inhibited DHT binding to TeBG. Thus, in our assay systems, compound I bound strongly to ABP but not to TeBG nor to the androgen receptor. These results, however, do not allow immediate predictions concerning binding of the nonsteroidal analogues to the controversial¹⁷⁻²⁰ human ABP as it is known that ABPs from different species differ in steroid specificity²¹.

The data presented here confirm that the androgen-binding sites on ABP and the androgen receptor have different specificities. We have found dicyclohexane derivatives which display an absolute specificity for ABP when tested in parallel for androgen receptor binding. Steroidal and nonsteroidal ligands which specifically inhibit binding of androgens to ABP may be of interest for studying regulation of male fertility.

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High-affinity binding of ¹²⁵I-labelled mouse interferon to a specific cell surface receptor

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Previous research suggests that interferon binds to the cell surface¹, possibly by attachment to gangliosides^{2,3}. A two-component receptor system consisting of binding and activation sites has been proposed⁴. This hypothesis is supported by the finding that interferon inhibits binding of cholera toxin and thyrotropin to their receptors^{5,6} suggesting possible common receptor sites. Moreover, an antiserum against cell surface components of interferon-sensitive cells has been shown to inhibit the action of interferon⁷. However, to understand the interaction of interferon with the cell surface requires direct ligand-binding studies. I present here direct evidence that high-affinity binding of interferon to a specific cell surface receptor is an initial step in interferon action using biologically active purified ¹²⁵I-labelled mouse interferon. Labelled interferon binds specifically to interferon-sensitive mouse leukaemia L1210 cells, whereas binding to interferon-resistant L1210 cells is nonspecific. Furthermore, specific binding to monolayer cultures of mouse L929 cells is compared with nonspecific binding to chick embryo fibroblasts insensitive to the action of mouse interferon⁸.

Partially purified mouse C243 cell interferon⁹ was further purified by affinity chromatography on a sheep-anti-mouse interferon antibody agarose column as previously described^{10,11}, followed by ion exchange chromatography on CM-Sepharose CL-6B (Pharmacia). This procedure yielded highly purified interferon with a specific activity of 5×10^8 - 10^9 mouse interferon reference units per mg of protein with a recovery of 50-75% (Fig. 1a). Interferon was assayed on mouse L929 cells challenged with vesicular stomatitis virus as described previously¹⁴; protein concentrations were estimated according to the method of Lowry.

Purified interferon was iodinated with ¹²⁵I to a specific radioactivity of 70-80 μ Ci per μ g of protein using a modified lactoperoxidase-catalysed reaction¹⁵; monoiodination of a protein with a molecular weight (MW) of 35,000 should result in a specific activity of 57 μ Ci per μ g of protein. Labelled interferon was reduced with 1% 2-mercaptoethanol in 0.1% SDS; this procedure allowed recovery of 25-50% of the initial biological activity, whereas the recovery was less than 20% before reduction, in agreement with similar previously reported results (ref. 16, Fig. 1b, c). Control experiments performed with cold iodine,

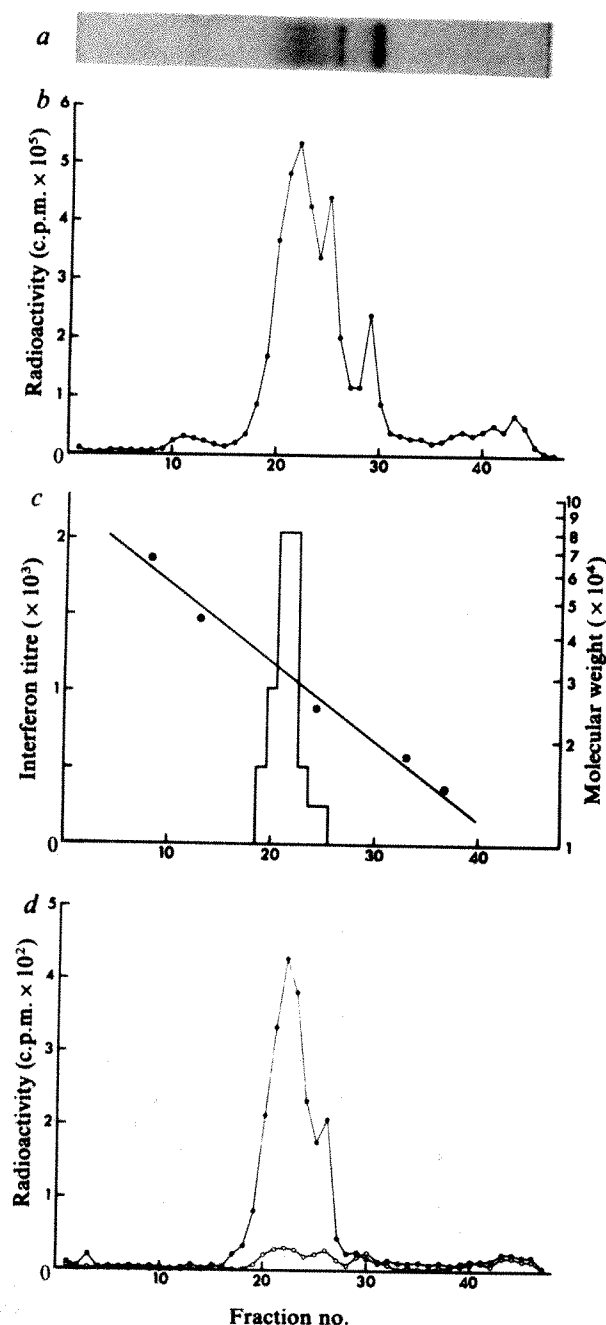


Fig. 1 SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli¹². Homogeneous slab gels of 12.5% acrylamide, 0.8 mm thick, 90 mm long and 130 mm wide were overlaid with a 10 mm long 3% stacking gel and run with a constant current of 10 mA in presence of 0.1% SDS (BDH). The samples were dialysed against 0.125 M Tris-HCl (pH 6.8) containing 1% SDS and heated for 2 min in a boiling water bath after addition of 1% 2-mercaptoethanol (Eastman). The sample size was 25 μ l. *a*, Coomassie blue staining of purified interferon. After elution from 2 mm gel slices, antiviral activity was associated with all three bands which correspond to apparent MWs of 35,000, 28,000 and 22,000. This interferon therefore appears to be electrophoretically similar to a recently described purified interferon prepared from Newcastle-disease-virus infected mouse Ehrlich ascites tumour cells¹³. *b*, Profile of radioactivity of ¹²⁵I-labelled purified interferon. The fractions correspond to gel slices of 2 mm. *c*, Profile of eluted antiviral activity from the material depicted in *b*. The profile is congruent with the radioactivity peaks at MWs of 35,000 and 28,000; however no antiviral activity was detectable at MW 22,000. Molecular weight markers (Sigma) were bovine serum albumin (66,000), egg albumen (45,000), trypsinogen (24,000), beta-lactoglobulin (18,400) and lysozyme (14,300). *d*, Profile of radioactivity of bound and eluted ¹²⁵I-interferon from L1210-S (●) and L1210-R (○) cells.

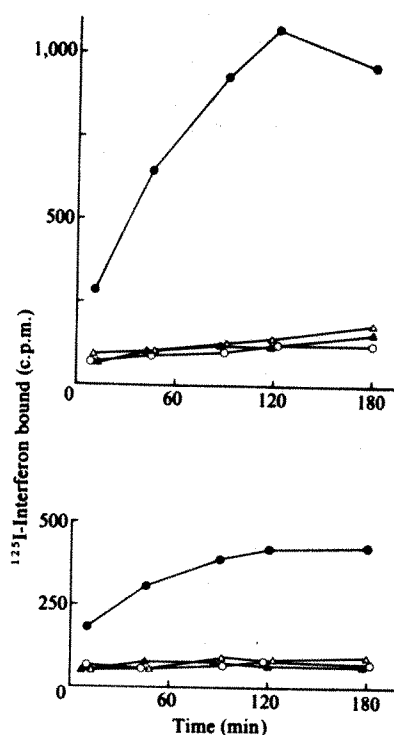


Fig. 2 The continuous cell lines of L1210-S and L1210-R cells were grown in non-agitated suspension cultures in RPMI 1640 medium (Flow laboratories) supplemented with 10% fetal calf serum (Gibco Bio-Cult)¹⁹. Cells (2×10^6) were incubated with ¹²⁵I-interferon in 24-well Costar 3524 plates at a density of 2×10^6 cells per ml and subsequently washed three times with culture medium by centrifugation at 4 °C. All experiments were performed in triplicate. The radioactivity was measured in a Gammatic gamma counter. *a*, Time-course of ¹²⁵I-interferon binding to L1210-S (●) and L1210-R (○) cells at a concentration of 400 U ml⁻¹ at 37 °C. Simultaneous addition of 4×10^5 U ml⁻¹ of unlabelled interferon inhibits binding to L1210-S (▲) cells, whereas binding to L1210-R (△) cells is unaltered; *b*, time course of binding at 4 °C. Standard deviations were less than 5%.

or omitting iodine in otherwise identical labelling conditions, suggest that the irreversible inactivation on iodination is due either to conformational changes as a consequence of substitution or to iodine-induced irreversible oxidation reactions.

Mouse leukaemia L1210 cells have been characterised extensively in terms of sensitivity to the various biological effects of interferon^{17,18}. An interferon-resistant subline of these cells has been cloned and the resistance to the different effects of interferon is well documented¹⁹. The time-course of ¹²⁵I-interferon binding to both cell lines is shown in Fig. 2: binding to interferon-sensitive L1210 cells (L1210-S) occurred rapidly and progressively and could be competitively inhibited by simultaneous addition of an excess of unlabelled interferon. Binding to interferon-resistant L1210 cells (L1210-R) was minimal and independent of the addition of unlabelled ligand. This binding can therefore be referred to as nonspecific. These findings confirm previous experiments on the recovery of antiviral activity from L1210-S cells, but not from L1210-R cells on treatment with large amounts of interferon¹⁹. While maximum specific binding was achieved after 120 min independently of temperature, the amount of specifically bound material was markedly higher at 37 °C than at 4 °C. We are now investigating whether this increased binding at 37 °C reflects a continuous uptake of interferon, as has been suggested previously²⁰.

The saturation of specific receptor sites for interferon is depicted in Fig. 3. Binding of ¹²⁵I-interferon to L1210-R cells as

a function of the concentration of added labelled ligand was linear and thus nonspecific; no saturation occurred in the range of biologically active concentrations. The specific receptor sites on L1210-S cells, however, were saturated between interferon concentrations of 3,200 and 6,400 units per ml, at 37 °C as well as at 4 °C (data not shown). Thus the saturation curve is congruent with the biological dose-response curve reported previously²¹.

To characterise this bound fraction further, L1210-S cells were incubated for 120 min with 6,400 units per ml of labelled interferon in the cold, washed and subsequently incubated with a 2,000-fold excess of unlabelled interferon for another 2 h at 4 °C. In these conditions 70% of the bound radioactive material was recovered, compared to less than 25% spontaneously released material. On analysis by SDS-PAGE the radioactivity profile of the specifically bound and eluted material corresponds to the profile of antiviral activity of the original ¹²⁵I-interferon preparation (Fig. 1c, d). Control experiments in which ¹²⁵I-interferon was bound and eluted from L1210-R cells confirmed the nonspecific binding to these cells (Fig. 1d).

The comparison of binding of ¹²⁵I-labelled mouse interferon to interferon-sensitive mouse monolayer L929 cells with binding to chick embryo fibroblasts, insensitive to mouse interferon⁸, yielded similar results to those obtained with L1210-S and L1210-R cells (data not shown). In contrast to the specific binding to mouse L929 cells binding of ¹²⁵I-labelled mouse interferon to chick embryo fibroblasts could not be inhibited competitively by simultaneous addition of an excess of unlabelled mouse interferon. Specific binding to mouse L929 cells revealed saturation at a concentration of 6,400 units per ml interferon and can thus be expected to occur with a similar high affinity as on L1210-S cells; however, chick embryo fibroblasts could not be saturated with ¹²⁵I-labelled mouse interferon in the same range of concentrations. Binding of mouse interferon to chick embryo fibroblasts could thus be characterised as nonspecific, in agreement with the observation that interferon can be recovered from homologous, but not from heterologous cells previously treated with high amounts of interferon²².

The various approaches for the characterisation of the specific interaction between high affinity membrane receptors and their ligands has been the subject of reviews by Kahn²³ and Cuatrecasas²⁴. The mass action law can serve as a model for the interaction of a ligand with its receptor on the assumption that the binding corresponds to a simple bimolecular equilibrium. It can be derived from the equation of mass action law, that the dissociation constant at half saturation of the receptor sites is equal to the concentration of free ligand at half saturation. As less than 10% of the added labelled interferon was bound to the cells at half saturation, the concentration of free ligand can be set equal to the known total ligand concentration. Assuming that labelled interferon possesses identical binding properties as the unlabelled material and that only biologically active labelled interferon binds specifically, an apparent affinity constant for binding of interferon to L1210-S cells can be derived from the saturation curve depicted in Fig. 3: half saturation is achieved at a concentration of 400–800 units per ml, equivalent to $1-2 \times 10^{-11}$ M for a mean MW of 35,000 and a specific activity of 10^9 units per mg protein.

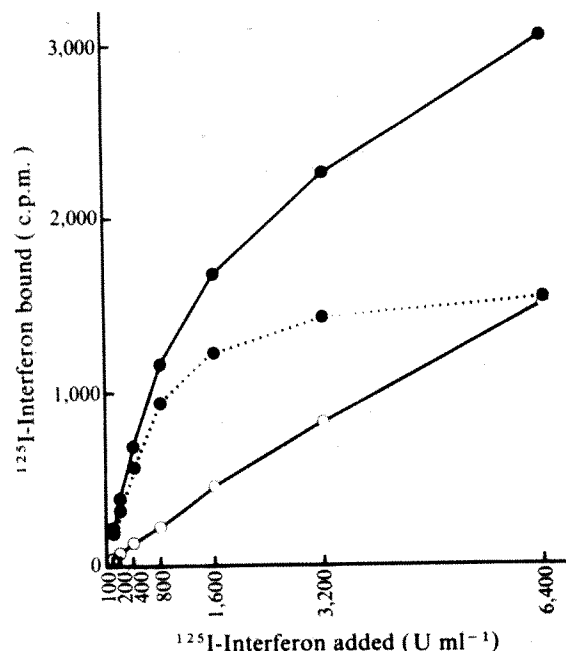


Fig. 3 Saturation of ¹²⁵I-interferon binding to L1210-S (●) and L1210-R (○) cells after 2 h incubation at 37 °C. The specific binding is depicted as the difference between total binding to L1210-S cells and nonspecific binding to L1210-R cells (dotted line). Standard deviations were less than 5%.

The present results provide, for the first time, direct evidence for high affinity binding to a specific cell surface receptor as the initial step in interferon action. Thus interferon binding shares a characteristic property of other biologically highly active substances such as peptide hormones and neurotransmitters²³. This strongly suggests that the mechanism of resistance to interferon across the barrier of species specificity, as well as the resistance of L1210-R cells is located at the cell surface. Ligand binding studies offer new possibilities for a direct approach to the study of several aspects of interferon action. Thus interferon antagonists, interferon derivatives or heterologous interferons may be analysed directly with regard to competitive binding and biological crossreactivity. Furthermore, use of biologically active, purified ¹²⁵I-interferon offers new possibilities to investigate binding and pharmacokinetic properties *in vivo*.

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Protein kinase in immunoprecipitate of DMBA-transformed epithelial cells with serum from tumour-bearing rabbits

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Recent work using virally transformed cells has shown that the viral gene product responsible for transformation is associated with protein kinase activity^{1,2}. Use of antisera specific for the *src* gene product of Rous sarcoma virus showed that the heavy chain of the antibody in the immunoprecipitate can be phosphorylated by the bound antigen *src* gene product. Only a very low level of such protein kinase activity was observed using uninfected cells or cells infected with transformation-defective virus. Such a low level of activity has been attributed to the endogenous *src* gene product^{3,4}. Similar protein kinase activity, capable of phosphorylating IgG, was also detected in the immunoprecipitate of the T antigen of adenovirus type 5 (ref. 5). Moreover, large T antigen of SV40 is also associated with protein kinase and/or ATPase activity^{6,7}. Thus, many of the virally coded proteins involved in transformation are apparently associated with protein kinase activity. In addition, secretion of specific phosphoproteins and increased protein kinase activity have been detected in conditioned medium from cells transformed by oncogenic viruses⁸. Secretion of these proteins, accompanied by an additional protein kinase, correlates well with the transformation as demonstrated by the use of temperature-sensitive mutants in the *src* gene of avian sarcoma virus. These data suggest that the alteration in protein phosphorylation may contribute to the transformed state. In the virion of murine sarcoma virus but not murine leukaemia virus, a novel protein kinase with molecular weight of 15,000 (15 K) has recently been discovered⁹. In view of these findings, it is of interest to investigate whether in chemical carcinogen transformed epithelial cell an analogous event also occurs. We studied transformed bladder epithelial cells induced after *in vitro* exposure to the chemical carcinogen, 7,12-dimethylbenz[*a*]anthracene (DMBA). Anti-serum against DMBA-transformed mouse bladder epithelial cells were raised in rabbits and used to immunoprecipitate antigens from chemically transformed cell lysates. By incubating the immune complex with [γ -³²P]ATP we found that a transformation-specific 18K protein becomes phosphorylated. Such a phosphorylatable 18K protein is also detected in the conditioned medium of DMBA-transformed cells.

Background work on the establishment, characterisation and tumorigenicity of the DMBA-transformed bladder epithelial cell lines has been published previously¹⁰. Tumours were induced by subcutaneous injection of DMBA-transformed mouse bladder epithelial cells ($1-2 \times 10^6$ cells) into 5-week old New Zealand rabbits. Three more weekly injections of the same cell line into the same site were given. One week after the last injection, blood was collected. Of the five antisera raised against different epithelial cell lines, immunoprecipitates from four gave identical results in the phosphorylation of an 18K protein. One of them, raised against MB49 and designated serum V, was used in this study because of its higher titre.

Cytoplasmic extracts prepared from cultures of primary cells, untransformed cell lines and chemically transformed epithelial cells, were immunoprecipitated with immune or non-immune serum on protein A-Sepharose beads. Extensively washed immunoprecipitates were then incubated with [γ -³²P]ATP, rewashed and analysed by SDS polyacrylamide gel electrophoresis. Figure 1e shows an immunoprecipitate of a DMBA-transformed epithelial cell line with phosphorylation of an 18K

protein which could sometimes be resolved as a doublet. Similar results were observed when the immunoprecipitate was made to a benzo[*a*]pyrene (BP) transformed rabbit bladder epithelial cell line. Non-immune serum (Fig. 1c,f) and a variety of other antisera against fibronectin, p60^{src}, FOCMA, SV40-T antigen and polyoma-T antigen, did not support a phosphotransfer reaction in the immunoprecipitate of the DMBA-transformed epithelial cells. We also failed to detect ³²PO₄-labelled 18K proteins in immunoprecipitates of immune serum V reacted with primary cultures of epithelial cells, fibroblasts and endothelial cells (Fig. 1h, i, j respectively). However, among a variety of established cell lines tested, the phosphorylation of the 18K protein was detected in immunoprecipitates of mouse BALB 3T3-A31 cells and CCL146 (gerbil fibroma) cells with immune serum V (Fig. 1b). In other established cell lines (Rat 1 and NRK) scored negative for protein kinase (Table 1), small amounts of radioactive incorporation in the 18K region could be detected (Fig. 1g) after prolonged exposure of the gel. Cell lines which failed to react with serum V to give the 18K protein included RK13 (rabbit kidney), CCL60 (rabbit fibroblast), CV-1 (monkey kidney) and RPL (rat liver). For virally transformed cells tested, no detectable phosphorylatable 18K was observed in immunoprecipitates with immune serum V in these experimental conditions. These include RSV-chick, RSV-rat (CCL47), SV80 (SV40 human cells), 64F3 (FeSV-CCL64) and T8 (adenovirus types 2 transformed rat cells). It should also be noted that the incubation of [γ -³²P]ATP with the immunoprecipitate of the *src* gene product of RSV or the T antigen of SV40 or polyoma virus does not result in the appearance of ³²P-labelled 18K protein.

When immune serum V was incubated with formaldehyde-fixed DMBA-transformed epithelial cells (MB49), and then

Table 1 Protein kinase activity in immunoprecipitates of different cell types with tumour-bearing serum

Cell types		Relative amount of ³² PO ₄ -labelled 18K protein, % of MB ₄₉
<i>Primary cultures</i>		
(1) Embryo fibroblasts	rat	0
(2) Embryo fibroblasts	chick	0
(3) Embryo fibroblasts	Chinese hamster	0
(4) Bladder epithelium	mouse	0
(5) Bladder epithelium	rabbit	0
(6) Aorta endothelium	cow	0
<i>Spontaneous cell lines</i>		
(1) CV-1	Monkey	0
(2) NRK	rat	5%
(3) Rat 1	rat	2%
(4) RPL	rat	0
(5) RK13	rabbit	0
(6) CCL 60	rabbit	0
(7) BALB 3T3-A31	mouse	80%
<i>Chemically transformed epithelial cell lines</i>		
(1) MB33 I	mouse-DMBA	100%
(2) MB33 II	mouse-DMBA	100%
(3) MB48 B	mouse-DMBA	100%
(4) MB49	mouse-DMBA	100%
(5) MB49 B	mouse-DMBA	100%
(6) MB63	mouse-DMBA	100%
(7) MB71 B	mouse-DMBA	100%
(8) RBC	rabbit-benzo[<i>a</i>]pyrene	100%
<i>Tumour-derived cell lines</i>		
(1) CCL146	gerbil-fibroma	100%
(2) B103	rat-neuronal tumour	0
<i>Virally transformed cell lines</i>		
(1) CCL47	rat-RSV	0
(2) 64F3	mink-FeSV	0
(3) SV80	human-SV40	0
(4) CEF/Ts68	chick-RSV	0
(5) T8	rat-adenovirus 2	0

used to immunoprecipitate antigens for chemically transformed cell lysates, phosphorylation of the 18K protein was greatly reduced (Fig. 1a, d). Removal of such activity can also be accomplished by incubation of antiserum with live DMBA-transformed cells. However, absorption of tumour-bearing serum V on fixed primary epithelial cells or cells scored negative for the phosphorylated 18K protein does not result in the reduction of phosphorylation activity after immunoprecipitation. Thus, it is reasonable to conclude that the phosphorylation activity observed in immunoprecipitate is induced after oncogenic transformation by the chemical carcinogen.

Antiserum V, although raised in the rabbit by injecting mouse cells, is not species specific. It recognises an 18K protein in mouse, rat, rabbit and gerbil cell lines. As summarised in Table 1, this 18K protein seems to be transformation-dependent. As the ^{32}P -labelled 18K protein is not detectable in immunoprecipitates of serum V with a variety of virally transformed cells, this protein is probably not a common transformation-dependent protein. Rather, it seems to be confined to only chemically transformed cells and a few immortal cell lines.

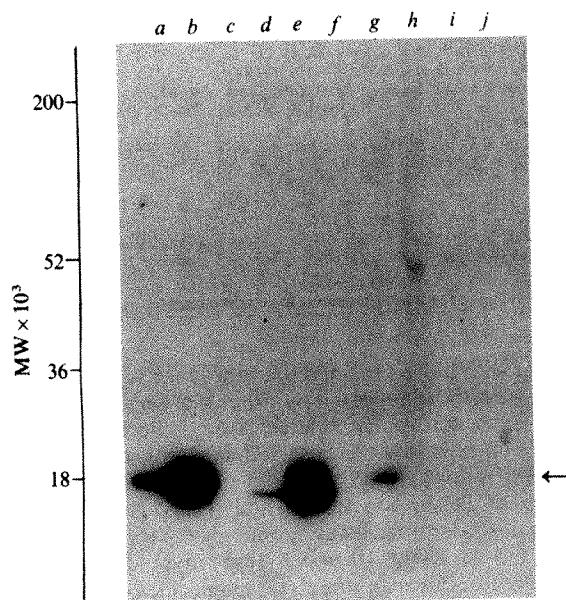


Fig. 1 Detection of a protein kinase activity in immunoprecipitates of various cell types. Cytoplasmic extracts were prepared and immunoprecipitated with tumour-bearing rabbit serum V, serum V absorbed with formaldehyde fixed DMBA-transformed mouse epithelial cells, MB49, or non-immune serum. Cells (2×10^6) were washed five times with STE buffer (0.15 M NaCl/0.1 M Tris-HCl, pH 7.2/0.001 M EDTA) and lysed in 0.7 ml of RIPA buffer (1.0% Triton X-100/1.0% sodium deoxycholate/0.1% SDS/0.15 M NaCl/0.01 M Tris-HCl, pH 7.2) supplemented with 200 units ml^{-1} of Trasylol (Calbiochem) at 4°C . Cell lysate (0.5 ml) was mixed with 10 μl of non-immune serum and 40 μl of protein A Sepharose CL-4B (Pharmacia). After 10 min of gentle agitation the mixture was centrifuged at 28,000g for 30 min and the supernatant was removed for immunoprecipitation. To 100 μl aliquots of supernatant 10 μl of immune serum 40 μl of protein A-Sepharose was added and incubated at 4°C for 30 min with constant mixing. The immune complexes were washed four times with RIPA buffer and then once with phosphate-buffered saline (PBS). The pellet was incubated at room temperature for 20 min in a buffer containing 0.1 M Tris, pH 7.4; 1 mM MgCl_2 and $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (0.1 μM , specific activity 2000 Ci mmol^{-1} ; NEN) and then washed four times with PBS to remove unbound radioactivity. The immune complexes were eluted from the Sepharose beads by boiling in buffer containing 1% SDS and 2% β -mercaptoethanol for 4 min. The supernatant was then analysed by SDS polyacrylamide (12.5%) slab gel. The gel was stained with Coomassie brilliant blue, dried and an autoradiograph was prepared by Kodak SB-5 film. Cell line derived from gerbil benign tumour, CCL146 with a, absorbed serum V; b, serum V; c, non-immune serum. DMBA-transformed mouse bladder epithelial cell line MB49 with d, absorbed serum V; e, serum V; f, non-immune serum; g, NRK with serum V. Primary cultures with serum V, h, mouse bladder epithelium; i, hamster embryo fibroblasts; j, bovine endothelial cells. The molecular weight markers used were myosin heavy chain (200K), tubulin (52K), troponin T (36K) and lactoglobulin (18K).

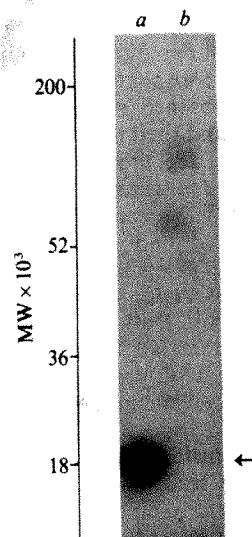


Fig. 2 Protein kinase in immunoprecipitates of conditioned medium from normal and chemically transformed cells. Conditioned medium immunoprecipitated with serum V, was incubated for 20 min with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ at room temperature. Immune complexes were eluted from Sepharose beads and analysed on an SDS polyacrylamide slab gel. Conditioned medium from a, DMBA-transformed epithelial cell line, MB49; b, primary culture of mouse bladder epithelium. The molecular weight markers used are myosin heavy chain (200K), tubulin (52K), troponin T (36K) and lactoglobulin (18K).

Serum-free medium was conditioned for 24 h with MB49 cells, collected from the dish and centrifuged to clear it of any cells. The conditioned medium was either incubated with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ for 20 min and then immunoprecipitated with serum V or immunoprecipitated first and then incubated with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. Both procedures resulted in the detection of ^{32}P -labelled 18K protein from DMBA-transformed cells (Fig. 2a) but not primary epithelial cells (Fig. 2b). The phosphorylation of an 18K protein in conditioned medium was destroyed when conditioned medium was first heated to 100°C for 1 min before incubation with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ but was unaffected by heat treatment at 56°C for 30 min.

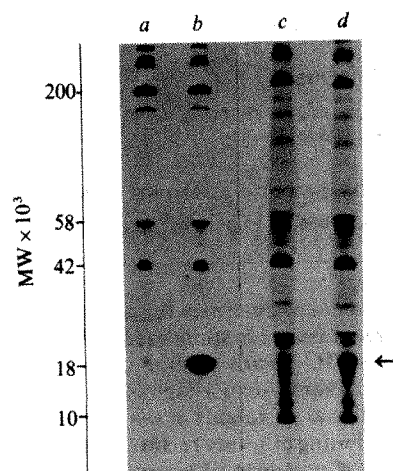


Fig. 3 SDS polyacrylamide gel analysis of immunoprecipitate of ^{35}S -methionine or ^{32}P -phosphate labelled conditioned medium from DMBA-transformed epithelial cell line, MB49, with tumour-bearing serum V. Confluent cells were incubated with ^{35}S -methionine (NEN, 400 Ci mmol^{-1} , 75 $\mu\text{Ci ml}^{-1}$) or ^{32}P -phosphate (NEN, 2,000 Ci mmol^{-1} , 200 $\mu\text{Ci ml}^{-1}$) in Dulbecco-modified Eagle's medium deficient in methionine or phosphate for 24 h. After centrifugation at 100g for 10 min, the supernatant was used for immunoprecipitation a, ^{35}S -methionine labelled, non-immune serum; b, ^{35}S -methionine labelled, tumour-bearing serum; c, ^{32}P -phosphate labelled, non-immune serum; d, ^{32}P -phosphate labelled, tumour-bearing serum. The molecular weight markers used were myosin heavy chain (200K), vimentin (58K), egg albumin (42K) and lactoglobulin (18K).

Evidence that the 18K protein is synthesised by cells and not acquired from serum was demonstrated by the incorporation of ^{35}S -methionine and ^{32}P -phosphate into 18K protein (Fig. 3) and the fact that in the absence of serum, cells continued to synthesise this protein and shed it into the culture medium.

Phosphorylation of the 18K protein, in immunoprecipitates of cell lysate or conditioned medium, was neither cyclic AMP nor cyclic GMP dependent. The reaction required a proper concentration of Mg^{2+} (1 mM) and was not inhibited by Ca^{2+} (1–10 mM). Intriguingly, it was inhibited by F-actin, similar to that of protein kinase associated with the *src* gene product¹¹ or with murine sarcoma virus⁹. Both ATP and GTP can be utilised for the phosphotransfer reaction. In many respects, the profile of phosphorylation of the 18K protein is similar to the phosphorylation of IgG heavy chain in the immunoprecipitate of *src* gene product.

The presence of a transformation-associated protein kinase activity reported in this study is similar, conceptually, to that reported for virally transformed cells^{1,2} but differs with respect to the substrate phosphorylated in similar experimental conditions. Serum directed against p60^{src}, the product of the *src* gene

of avian sarcoma virus, does not immunoprecipitate any detectable kinase activity from DMBA-transformed cells and conversely, immunoprecipitates of serum V with virally transformed cells does not result in the phosphorylation of the heavy chain of IgG on addition of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. Since alteration of protein phosphorylation plays an important part in several biological processes^{12–14}, the difference found between normal and chemically transformed cells in this respect may be of significance to the understanding of transformation induced by chemical carcinogens.

The possibility that our transformation-associated phosphoprotein may have growth-promoting properties¹⁵, protease activity¹⁶ or angiogenesis activity¹⁷ are currently under investigation. The isolation of the 18K phosphoprotein should help identify its biochemical role and clarify as to whether the expression and secretion of this protein is a causative or secondary event in transformation induced by chemical carcinogens such as DMBA or BP.

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Characterisation of an adrenal zona glomerulosa-stimulating component of posterior pituitary extracts as α -MSH

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The secretion of aldosterone from the zona glomerulosa of the mammalian adrenal cortex is stimulated by ACTH, potassium, angiotensin II and III, growth hormone, serotonin and E series prostaglandins^{1–7}. Some experimental and clinical studies suggest that additional stimulants of the zona glomerulosa must exist, possibly including pituitary factors other than ACTH^{6,8–13}. The possibility that posterior pituitary extracts may contain a zona glomerulosa stimulant was first suggested 20 years ago^{14,15}, but has since received little attention. We describe here the purification from posterior pituitary extracts of activities that stimulate rat glomerulosa cells and whole tissue *in vitro*. One of the active compounds has been identified as α -MSH (melanocyte stimulating hormone).

Two posterior pituitary extracts were investigated, one an extract of bovine posterior pituitaries¹⁶, the other Pitressin (Parke Davis). Their effects on the production of corticosterone by adrenal cell suspensions compared to ACTH are shown in Table 1. Values obtained for the stimulation by ACTH are

consistent with those obtained previously in these laboratories, and also with those reported by other workers^{1,17,18}. Posterior pituitary extract 1 also stimulated both glomerulosa and inner zone adrenal cells, but the dose required to give half-maximal stimulation is lower than expected on the basis of immunoassayable ACTH by a factor of about 17 for glomerulosa cells based on the C-terminal assay¹⁹, but only 6.6 for the N-terminal assay¹⁹, and of about 10 and 3 respectively for fasciculata/reticularis cells. Pitressin stimulates glomerulosa cells alone and has no effect on inner zone cells. Neither vasopressin, substance P nor neurotensin have any effect on either cell type in the conditions used.

For bioassay purposes, the production of corticosterone alone was used as a measure of steroidogenesis. However, the effect of the stimulators on the total steroid profile of the glomerulosa using whole tissue preparations is shown in Fig. 1. It can be seen

Table 1 Potencies of various stimulants on rat adrenocortical cell suspensions

	ACTH (mol l ⁻¹)	Posterior pituitary extract no. 1 (μg)	Pitressin (pressor units)
Glomerulosa cells	6.6×10^{-11}	13.7 (4×10^{-12} C-terminal; 4×10^{-11} N-terminal)	108 mU
Fasciculata/ reticularis cells	6.9×10^{-11}	20 (6×10^{-12} C-terminal; 1.5×10^{-11} N-terminal)	NS

Values are amounts of stimulant which in standard incubation conditions give half maximal stimulation of corticosterone production. Units are: ACTH, mol l⁻¹; posterior pituitary extract 1, μg per flask (values in parenthesis are expected ACTH contamination as measured by two radioimmunoassays¹⁹, in mol l⁻¹); Pitressin, pressor units per flask. NS; no stimulation.

that qualitatively similar results were obtained with ACTH, posterior pituitary extract 1, and Pitressin. Corticosterone and 18-hydroxycorticosterone (18-OH-B) are most stimulated, with a smaller increment in aldosterone. The effect on deoxycorticosterone (DOC) is variable, while 18-hydroxy-DOC (18-OH-DOC) is not affected. The response of the steroid profile in intact (non-dispersed) tissue is highly characteristic and contrasts with the effects of potassium ions, or dibutyryl cyclic-AMP, both of which stimulate 18-OH-DOC as well as the other products^{20,21}.

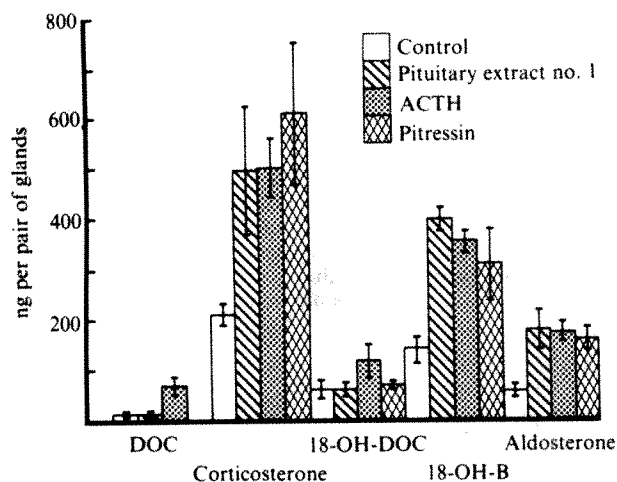


Fig. 1 Profile of steroids obtained during incubation of rat adrenal zone glomerulosa (intact) in control conditions and with the addition of ACTH ($1 \mu\text{g}$ per flask, approximately $5.5 \times 10^{-8} \text{ mol l}^{-1}$), crude posterior pituitary extract 1 ($100 \mu\text{g}$ per flask, approximately $1.5 \times 10^{-7} \text{ mol l}^{-1}$ α -MSH/ACTH as judged by the N-terminal assay) or Pitressin (2 pressor units per flask, approximately $0.6 \times 10^{-7} \text{ mol l}^{-1}$ α -MSH) to the incubation flasks. A characteristic of all these stimulants is that corticosterone, 18-OH-B and aldosterone are stimulated ($P < 0.05$ or better) whereas 18-OH-DOC is not. This contrasts with stimulation by potassium or dibutyryl cyclic AMP, which also stimulate 18-OH-DOC. Values are means \pm s.e.m., $n = 6$ throughout. Male rat adrenals were used for incubation of whole tissue. Pairs of capsules which consist mostly of zona glomerulosa cells but with 5–8% contamination with fasciculata cells were incubated in individual flasks containing 5 ml Krebs–Ringer (without bovine serum albumin (BSA) or collagenase, see legend to Fig. 2). After 1 h preincubation the medium was discarded and the tissue resuspended in fresh Ringer. Incubations, to which stimulants were added, were for 2 h.

Fractionation of Pitressin was achieved using reverse-phase high pressure liquid chromatography (HPLC) in a salt-free system²² (Fig. 2, a similar profile of this preparation has been reported for a salt-based system²³). Assay for glomerulosa cell stimulation shows the activity mostly to be associated with three UV absorbing peaks indicated on the chromatogram (peaks A–C). When these are pooled and tested against unfractionated Pitressin at a range of concentrations, the dose–response curves show that at least 70% of the total glomerulosa stimulating activity in Pitressin is contained in these fractions. From sample to sample of Pitressin, however, their relative amounts vary enormously, even between vials of the same batch. In some samples the UV absorbing peaks are absent altogether, and in these the eluates from the HPLC columns are also devoid of glomerulosa-stimulating activity. Rechromatography of peak A (or B) gives a single peak in the ultraviolet co-incident with biological activity.

Peak A was further characterised in the following ways:

(1) Treatment of the active sample with leucine aminopeptidase and, separately, carboxypeptidase Y did not lead to destruction of the biological activity. However, treatment with elastase destroyed the biological activity and, overall, these data suggested the presence of a peptide blocked both at the N- and C-termini.

Table 2 Amino acid content of tubes 26 to 31 after chromatography of 1 ml of Pitressin on the preparative HPLC column (see legend to Fig. 2)

	Fraction no.					
	26	27	28	29	30	31
Asx	2.7	1.2	3.1	3.4	3.5	3.5
Thr	1.0	0.7	2.0	2.0	2.1	1.7
Ser	2.0	2.1	4.8	6.0	3.4	2.6
Glx	4.3	3.4	6.2	7.1	5.5	4.9
Pro	4.3	1.6	7.6	8.9	5.0	5.2
Gly	3.9	2.2	5.4	6.3	4.8	4.6
Ala	3.2	1.5	3.4	4.1	4.1	4.5
Val	1.7	1.2	2.8	4.3	2.8	2.2
Met	0.6	0.5	1.9	2.4	1.0	0.5
Ile	0.4	0.6	0.6	0.7	0.8	0.8
Leu	1.8	0.8	2.2	2.8	3.2	2.9
Tyr	1.4	0.9	2.3	3.0	1.7	1.0
Phe	2.0	1.4	3.0	3.8	2.3	1.6
His	1.1	1.2	2.3	3.4	1.5	1.2
Lys	1.1	1.0	2.2	2.8	1.6	1.2
Arg	1.1	1.1	2.7	3.5	1.8	1.8

Peaks A and B correspond to tubes 28 and 29 respectively, and a tentative amino acid analysis for peak A is obtained by subtraction of 'blank' tubes (26, 27, 30 or 31) from tube 28. Values are in nmol ml^{-1} .

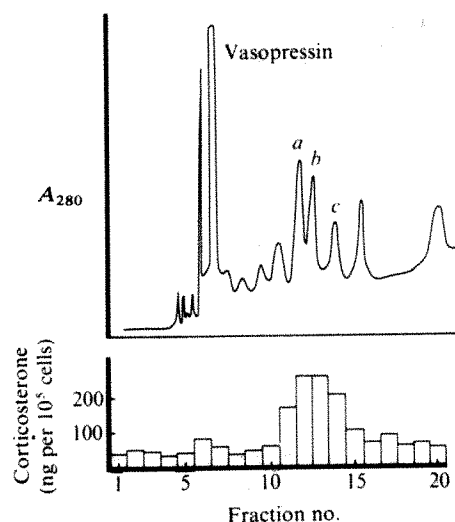


Fig. 2 UV profile of reverse-phase HPLC fractionation of Pitressin using a $0.39 \times 30 \text{ cm}$ column and the conditions given in ref. 22. A similar UV profile was obtained using a $0.78 \times 30 \text{ cm}$ column in the reported conditions²². The effect on corticosterone synthesis of adding these fractions to incubations of glomerulosa cells is shown in the lower part of the figure. For bioassays Wistar strain rats from the colony maintained at St Bartholomew's Medical College were used. Animals were killed by cervical dislocation, and the adrenals were quickly excised and stored on ice for brief periods before incubation. The glands were decapsulated and the capsules, containing largely zona glomerulosa cells (approximately 7% contamination by inner zone cells) and inner zones (zona fasciculata and zona reticularis) were incubated separately in Krebs bicarbonate Ringer solution (3.6 mM K^+) with 1% BSA (Sigma, fraction V), 200 mg glucose per 100 ml (KRBGA), and 2 mg ml^{-1} collagenase (Worthington). Tissue from 20 animals was incubated in 20 ml medium for 1 h at 37°C under 95% O_2 , 5% CO_2 . The tissue was then disrupted by repeated pipetting, filtered through nylon gauze, and centrifuged at 100g for 10 min. Cells were resuspended in 2 ml medium, and recentrifuged. The supernatant was discarded and the cells resuspended in a volume of incubation medium suitable for dispensing to incubation flasks.

(2) Amino acid analysis of column fractions spanning the biological activity (tubes 26–31) gave the data shown in Table 2. Subtraction of the non-active 'blanks' from the active fractions leads to a tentative conclusion that the active peptide is approximately 15 residues long with 1 Lys and 1 Arg or 30 residues long with 2 Lys and 2 Arg and so on. (3) Mass spectrometric

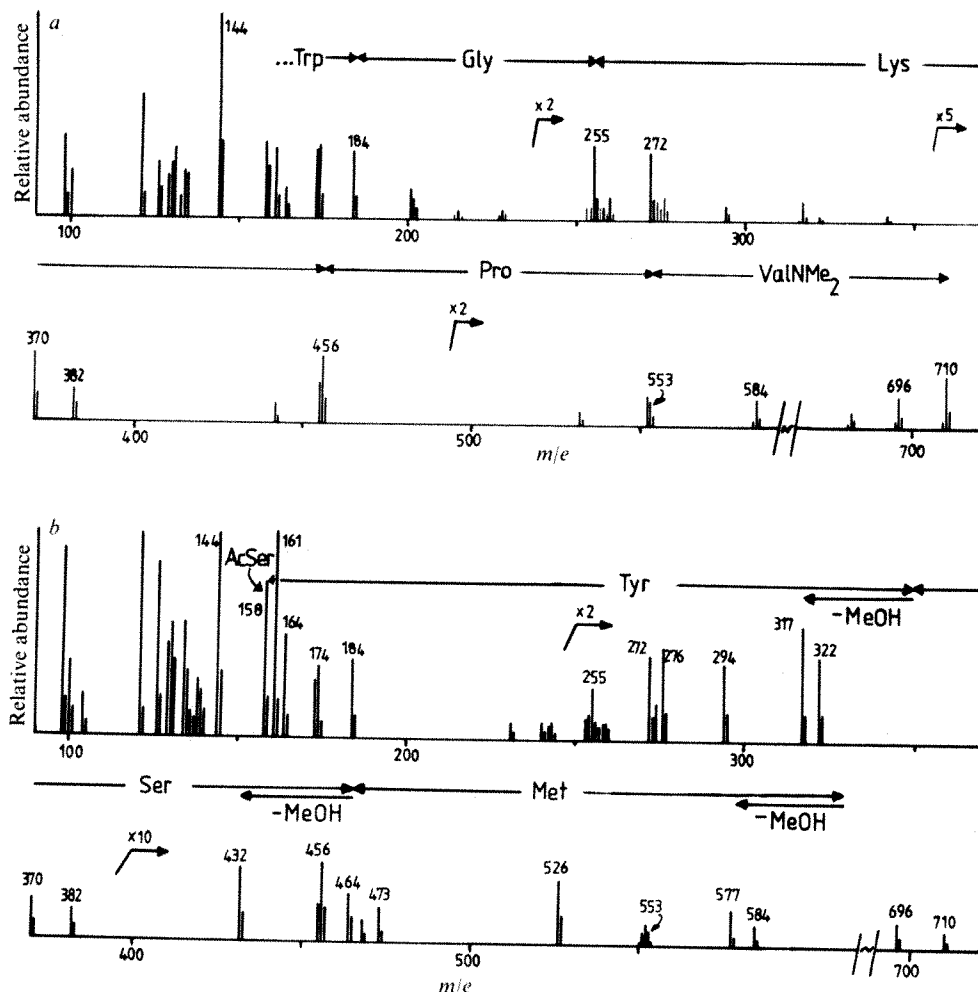


Fig. 3 Electron impact mass spectra of a tryptic digest of peak A at an ion source temperature of 300 °C (a) and at an ion source temperature of 350 °C (b). Spectra were recorded on a Kratos MS 50 mass spectrometer operating with an accelerator voltage of 8 kV and an electron beam energy of 70 eV. Before mass spectrometric examination peak A was dissolved in 100 μ l of 50 mM ammonium bicarbonate and treated with trypsin (1 μ g) for 3 h at 37 °C. After freeze drying the digest was deuterioacetylated and permethylated according to published procedures^{24,25}.

examination after trypsin digestion and derivative formation^{24,25} yielded good spectra; two representative spectra of the active material are shown in Fig. 3. The blocked N-terminus of the peptide is clearly established as acetyl-Ser-Tyr-Ser-Met- via signals at m/e 158, 317, 432, 464, 577 (Fig. 3b) and the blocked C-terminus is identified as Trp-Gly-Lys-Pro-Val-NH₂ via 'N-C cleavage' signals²⁶ at m/e 184, 255, 456, 552, 553, 696, 710 (Fig. 3a). Signals corresponding to an N-terminal Trp sequence, in addition to the N-C cleavage fragments assigned in Fig. 3, were observed in several spectra obtained during the source temperature gradient used for the examination of the tryptic digest of peak A. This suggests that the tryptophan is preceded by Lys or Arg (Arg if there is only one residue of each). Signals at m/e 98, 131, 134, 135 and 138 indicate the presence of glutamic acid, phenylalanine and histidine in the sequence of the molecule, and the lack of additional peptide signals in the spectra suggests that the peptide is likely to be approximately 15 rather than 30 residues in length. Overall the structural data obtained from the mass spectra may be summarised as

(Acetyl-Ser-Tyr-Ser-Met- (Glu, Phe, His) -Arg-Trp-Gly-Lys-Pro-Val-NH₂)
 approximately 15 residues

Comparison of these data with known structures of biologically active peptides suggests the probability that the active component of Pitressin is α -MSH:

Acetyl-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-NH₂,

which has identical N- and C-terminal sequences and whose structure is consistent with the other mass spectrometric and amino acid analysis data.

Accordingly, synthetic α -MSH was chromatographed both separately and together with Pitressin and found to co-elute with

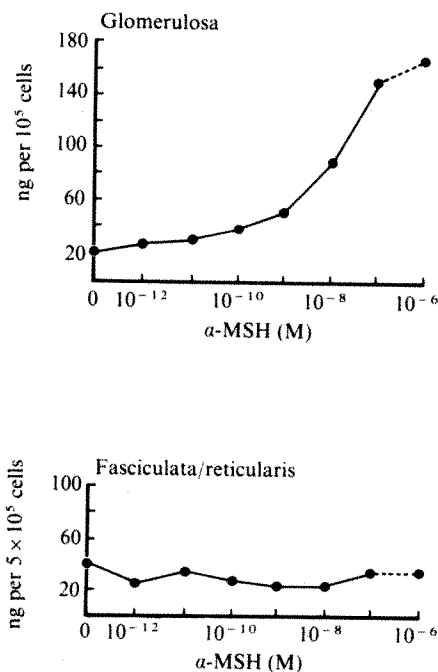


Fig. 4 Effects of α -MSH on rat adrenocortical cells (dispersed, see legend to Fig. 2) as judged by corticosterone production, at various concentrations. All values are means of two observations in a single experiment (that is, a uniform cell crop) except for dotted extrapolations which are based on data derived in a separate experiment.

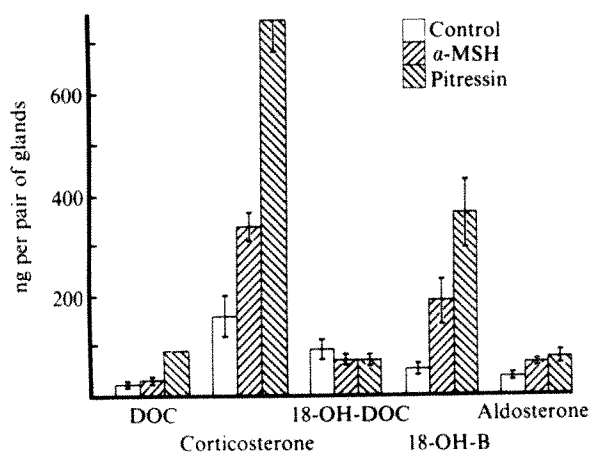


Fig. 5 Profile of steroids obtained during incubation of rat adrenal zona glomerulosa (intact tissue, see legend to Fig. 1) in control conditions and with the addition of α -MSH ($0.5 \text{ nmol per flask}$, $10^{-7} \text{ mol l}^{-1}$) and Pitressin (4 pressor units, containing approximately $0.6 \text{ nmol } \alpha\text{-MSH}$, $1.2 \times 10^{-7} \text{ mol l}^{-1}$). Qualitatively the results obtained with the two stimulants are similar in that corticosterone, 18-OH-B and aldosterone are all stimulated ($P < 0.05$ or better) whereas 18-OH-DOC is not; effects on DOC are variable. These results parallel those obtained with ACTH and posterior pituitary extract 1 (see Fig. 1). Quantitative differences between the two stimulants are attributable to the inclusion in Pitressin of further peptides (B and C, see Fig. 2) which also stimulate the rat zona glomerulosa cell. Values are means \pm s.e.m., $n = 6$ throughout, except for Pitressin-stimulated DOC, $n = 2$.

peak A. Furthermore, examination of peak A on HVPE gives, on staining with Cd-ninhydrin²⁷, a major spot with identical mobility to α -MSH, in addition to more weakly staining neutral and acidic peptides. Overall, the structural and chromatographic data strongly indicate that the biological activity is derived from α -MSH.

Authentic α -MSH was therefore tested in both types of incubation system (Figs 4 and 5). Clearly the features of the response to α -MSH show many points in common with the response to Pitressin. In the first place, only glomerulosa cells are stimulated, and there is no effect on the fasciculata/reticularis cells (shown to respond normally to ACTH). Second, the pattern of the intact capsule steroid profile and its response to stimulation is similar to Pitressin (and to ACTH, see Fig. 1) in that corticosterone, 18-hydroxycorticosterone and aldosterone were stimulated, whereas DOC and 18-OH-DOC were not affected. Half maximal stimulation with synthetic α -MSH is obtained at about $10^{-8} \text{ mol l}^{-1}$ and with Pitressin at 100 mU per flask (Table I) which from its calculated α -MSH content would

yield about $3 \times 10^{-9} \text{ mol l}^{-1}$ of α -MSH. It seems likely from this data that the stimulatory potential of Pitressin is partly, perhaps 30%, due to α -MSH. In the case of posterior pituitary extract 1 it is also likely that the greater sensitivity of glomerulosa cells (compared with fasciculata/reticularis) to its stimulation may also be due to α -MSH in part, since the N-terminal ACTH assay, with which there is a 100% cross-reaction with α -MSH¹⁹ gave higher values than the C-terminal assay. However, it is also certain that this extract contained intact ACTH and possibly other factors to account for its greater potency when compared with Pitressin on the basis of its N-terminal reactivity (Table 1).

Earlier studies have suggested the stimulation of aldosterone production by α -MSH^{28,29}, and recently it has been shown that cortisol production by fetal human and sheep adrenal cells responds to α -MSH stimulation but this capacity is lost in the adult sheep^{30,31} (although conflicting results have been obtained with rhesus monkey fetal adrenal cells³²). It seems likely therefore that although the major site of steroidogenesis in the adult adrenal, the fasciculata cell, is not responsive to α -MSH, other cell types may retain this capacity.

Whether α -MSH functions as a trophic hormone on the zona glomerulosa in the adult is a matter for further investigation. Reported circulating levels of α -MSH in normal rats are of the order of $10^{-10} \text{ mol l}^{-1}$, below the threshold of stimulation in our experiments (see Fig. 4)³³. However, plasma levels will certainly vary, depending on the physiological condition of the animal, and in this connection it is particularly interesting to note that changes in sodium status in rats exert profound effects on pars intermedia histology and α -MSH content, and thus presumably on α -MSH secretion³⁴⁻³⁶. While it might be expected that a true physiological messenger should (in contrast to the effect of α -MSH) elicit a greater response on aldosterone than on corticosterone, experience with other stimulants such as potassium or angiotensin II added acutely has shown that such 'late pathway' effects are only revealed with the adoption of a harsher methodology (see for example ref. 37). It is also possible that there may be species variation in the response of the zona glomerulosa to MSHs in view of the relative lack of response of the human gland to α -MSH³⁸ and of the sheep gland to β -MSH³⁹.

The identities of the remaining UV peaks (B and C) which are coincident with biological activity are now being determined using the combination of purification and mass spectrometric methods described above.

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A newly identified population of presumptive microneurons in the cat retinal ganglion cell layer

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A large population of microneurons has recently been discovered in the rabbit retinal ganglion cell layer^{1,2}. These 'coronate' cells may represent a class of displaced amacrine cells^{1,2}. Like conventional amacrine cells³ they are swiftly and selectively destroyed⁴ by low concentrations of kainic acid, a neurotoxin⁵. No similar cell has been described in the cat retina, which is reported to contain 217–260,000 neurones of classical appearance⁶. These outnumber the 128–180,000 optic nerve fibres^{7,8} but it has been suggested that the excess comprise Nissl-staining glial cells⁹. We report here that, using the neurotoxic effects of kainic acid to test and confirm the neuronal nature of the classic neurone excess, a large additional population of at least 730,000 presumptive microneurons was revealed. They resemble rabbit coronate cells, do not project into the optic nerve and have been previously identified as presumed glia^{6,10,11}. Various lines of evidence for the neuronal nature of these cells is presented below, but synapses have not been demonstrated; subsequent reference to microneurons must therefore be regarded as presumptive.

The vitreous humor of cats anaesthetised with Nembutal was injected with either 12 or 120 nM of kainic acid in 10 μ l of saline. After 3 h the retina was removed, mounted and stained⁶.

As shown in Fig. 1, near the area centralis, α , β and larger γ ganglion cells¹² seemed to be normal at the lower dosage but, apart from one microglial cell, none of the remaining non-vascular profiles resembled those of normal retina, and all showed kainic-induced necrocytosis; the nuclei of many were pyknotic⁵. Small γ -mode neurones seemed scarce but the number of necrotic profiles was far in excess of their possible

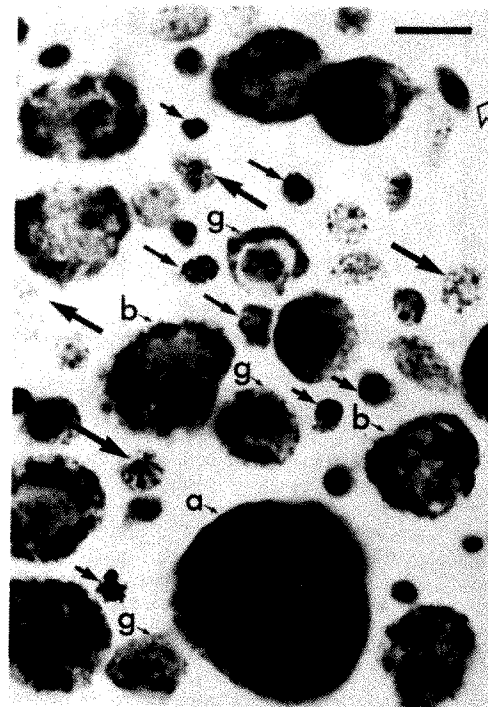


Fig. 1 Cells in the ganglion cell layer of the cat retina after an intraocular injection of 10 nM kainic acid. They are situated near the 2,500 classic neurones mm^{-2} isodensity line some 1.5 mm temporal of the area centralis. α (a), β (b) and the larger γ (g) Nissl-stained somas are apparent in relatively unchanged form. One γ cell near the centre of the figure has begun to degenerate and shows the characteristic nucleus with clumped chromophilic material. The large, medium and small arrows indicate profiles in progressively more advanced stages of degeneration. The final product is the pyknotic profile designated by the smallest unlabelled arrows. The open arrow indicates a classic glial cell. The degenerating profiles of this region must represent small cells because the densities of large and medium-sized cells are normal. The number of kainic-sensitive cells far exceeds the possible number of degenerate γ cells. Alcohol/formalin-fixed material. Scale bar, 10 μ m.

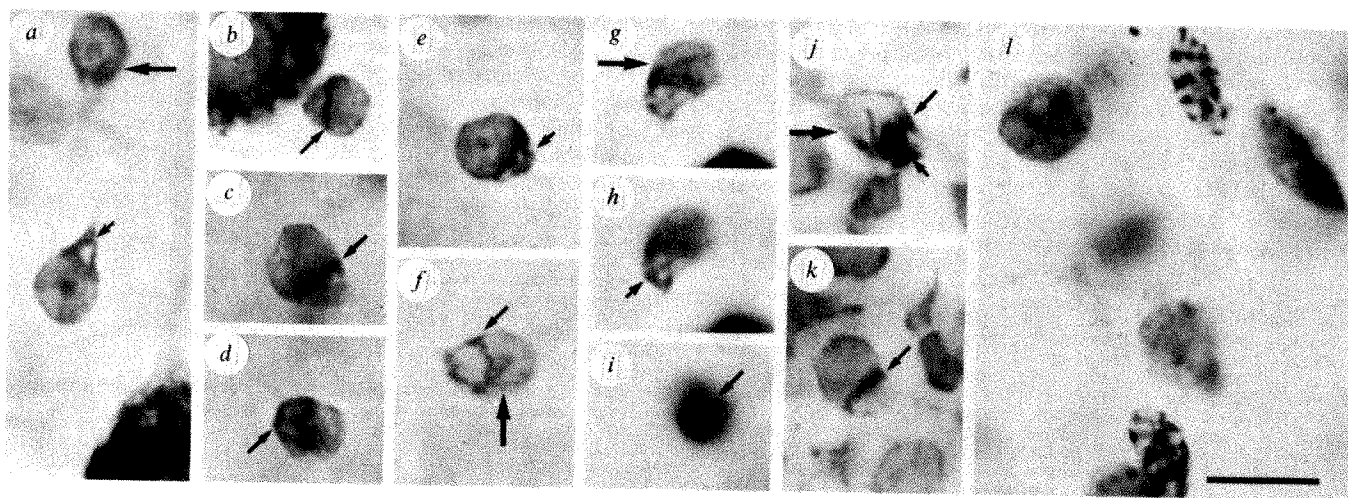


Fig. 2 a–i, Cells from the untreated cat ganglion cell layer resembling those which are destroyed by kainic acid and have previously been presumed to be glia. Small arrows: RNA-specific basophilia in free cytoplasmic granules; medium arrows: similar material at the juxtannuclear region of the cell's cytoplasmic pole; large arrows: basophilic nuclear invaginations. The nucleolus is obvious in cells of a and e. The subnuclear ring of basophilia is apparent in d, f and i; in i it is below the nucleus. j, k, Cells of the amacrine layer with features resembling those of dispersed, chromatin. These are from an eye injected with 120 nM of kainic acid and enucleated after 3 h, but their appearance is within the normal range. Cells such as those in a–k were destroyed by this treatment although they lie deeper within the retina. Alcohol/formalin fixed. Scale bar 10 μ m.

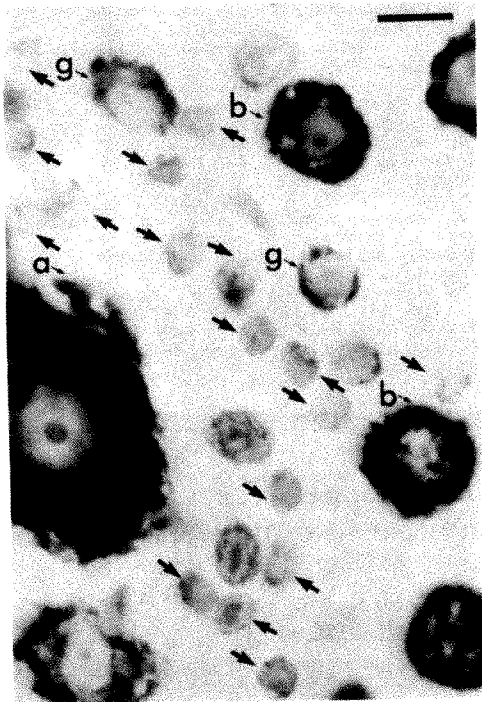


Fig. 3 A lightly Nissl-stained region of normal retina somewhat more peripheral than that of Fig. 1. The somas of Nissl-stained α (a), β (b) and γ (g) mode neurones are apparent. The small arrows indicate the profiles of cells previously identified as presumed glia^{7,12,13}. A large number of these cells are present, as would be expected in a region of normal retina similar to that shown in Fig. 1. However, no such profiles with relatively clear nuclei and juxtanuclear basophilia can be seen in Fig. 1. A quantitative analysis (see text) shows the majority of the kainic-sensitive profiles to correspond to the small, pale profiles shown here. These presumed glia^{7,13} are thus sensitive to the influence of a neurotoxic compound not known to have gliotoxic effects. Alcohol/formalin-fixed material. Scale bar 10 μ m.

contribution; the surprisingly large population of pyknotic cells was particularly obvious in the periphery, where ganglion cells are less common.

A 0.013-mm² area near that shown in Fig. 1 contained 75 non-vascular profiles. Of these, 2 were classic glia with obvious nuclear chromatin, 2 were α -mode cells, 15 β -mode cells and 8 γ -mode cells; the remainder were unlike any profiles of normal retina. Because this region in untreated retina has a γ -cell population equal to the combined α - and β -mode populations¹³ it is estimated that nine of the novel profiles represent degenerate classic neurones of the γ mode. Thus, 34 profiles (45%) represent classic neurones; 2 (3%) were classic glia and the remaining 39 (52%) represent cells not previously included in the neurone count but which have been destroyed by a neurotoxic compound.

In normal retina this area (Fig. 5A of ref. 6) contains a similar proportion (43%) of classic neurones, but the remaining 57% of profiles are presumed to be glia. The most common of these 'presumed glia' lack the conspicuous nuclear chromatin of classic glia and often possess a prominent nucleolus⁶. In kainic-treated retina only 3% of profiles in the ganglion cell layer were recognisable as glia; the remaining 54% of presumed glia were missing. There was instead a corresponding proportion of 52% residual kainic-damaged profiles which we conclude represents the necrotic presumed glia of the normal cat retina; their absolute densities are similar at 2,800 mm⁻² and 2,960 mm⁻² (ref. 6).

Kainic acid does not seem to be gliotoxic¹⁴; early glial response to kainic acid^{15,16} resembles that to glutamate^{3,17,18} and is reported to involve swelling but not necrosis and pyknosis. The exposed glial cells of the fibre layer and perivascular glia were unchanged in appearance even at the 10 times higher dose of kainic acid (Fig. 2I), as were glia in the ganglion cell and inner plexiform layers. Müller cell bodies and feet seemed normal, without the damage we observe with DL-amino adipic acid, a known gliotoxic compound¹⁹. On this basis, we conclude that the majority of the presumed glia in the normal cat ganglion cell layer are, in fact, neurones.

Figure 3 shows a lightly stained region of normal cat retina; the majority of profiles correspond to the presumed glia of previous reports^{6,9,11}. The pear shape of some cells (Fig. 2), the juxtanuclear basophilia, basophil nuclear invaginations and

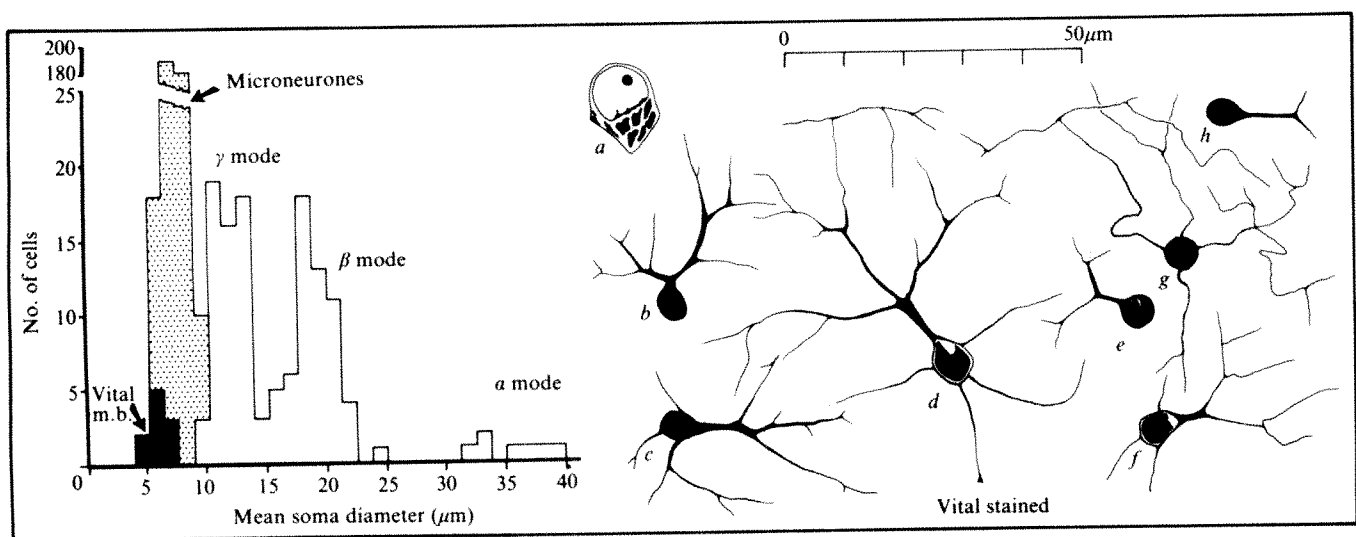


Fig. 4 The soma diameter spectrum illustrates the size range of non-vital stained cells in the various classic neurone modes of peripheral cat retina subject to supravital methylene blue staining and non-vital counterstaining. In addition, the stippled regions represent the presumed microneurone spectrum previously identified as presumed glia. The location in the soma size spectrum of some supravital methylene blue-stained microneurones is indicated in black. The partially stained unequivocal neurones *b* to *h* occupy the lower range of the microneurones. A medium-sized γ soma is shown at *a* for comparison. Neurones occupying a region of the diameter spectrum significantly outside the γ mode of classic neurones have not previously been reported. Their presence shows that cells in the class labelled 'microneurones' are not too small to be neurones of the cat retinal ganglion cell layer.

cytoplasmic granules, size range and restricted cytoplasm are similar to those of rabbit coronate cells². Some possess the 'crown' of juxtanuclear basophilia which led to the term 'coronate' in rabbit (Fig. 2*d, f, i*); its basophilia to Galloxyanin-lake is confirmed to be RNA-specific in the cat by its elimination with prior ribonuclease incubation, a result similar to that seen in the rabbit². The complex nuclear form^{20,21}, its basophilic invaginations²¹ and cytoplasmic RNA basophilia²² are characteristically neuronal^{20,21}.

These cells are smaller than any neurones previously described in the cat retinal ganglion cell layer and are different from reported cat displaced amacrine cells²³. Supravital methylene blue-stained retinas were examined for examples of their dendritic trees. Figure 4 shows the diameter range of soma profiles for neurones in such preparations. Dendritic trees are shown only for cells with very small, 5–7 µm, somata well displaced from the γ-mode population. None are completely stained but the variety of form suggests considerable heterogeneity; their vital staining offers additional evidence for their neuronal nature²⁴. Many possess one main dendrite which branches close to the soma. No axons or axon-hillocks were observed and the cells do not fill with horseradish peroxidase from the optic nerve¹³.

In peripheral retina the presumptive microneurones form 75% of the profiles in the retinal ganglion cell layer but their density is lower, 1,550 mm⁻². In a 470-mm² cat retina⁶ this suggests a population of at least 730,000 microneurones. Adding the 217,000 classic neurones⁶ we estimate a population of up to 10⁶ neurones in the cat ganglion cell layer. This is so far in excess of even the largest optic nerve fibre counts that it precludes the possibility of a significant proportion of the microneurones being ganglion cells. That such a large population has remained undiscovered for so long suggests that they are, like the interplexiform cell²⁵, only rarely impregnated by the capricious Golgi stain.

Two classes of neurone which do not project into the optic nerve are recognised in the retinal ganglion cell layer—cells of Marengi^{26,27} and displaced amacrine cells²⁸. Cells of Marengi, unlike the microneurones of cat and rabbit retina, possess an axon. However, cells similar to microneurones do occur in the amacrine layer (Fig. 2*j, k*) of both species. Rabbit coronate cells², cat microneurones and non-displaced amacrine cells³ are all sensitive to kainic acid. It is concluded that the microneurones represent a class of 'displaced' amacrine cell. There are additional reasons for assuming this in the rabbit².

Displaced amacrine cells are regarded as common in non-mammalian species²⁸ but have only occasionally been identified in mammals^{23,28–30}. Their large number in the bird retina^{31,32} has been well demonstrated³³. Now cat, rabbit^{1,2} and rat³⁴ are also known to possess a large population of neurones in the ganglion cell layer which are not ganglion cells. No distinctive role has been postulated for either displaced amacrine cells or the microneurone population. The relative uniformity of microneurone density across the retina suggests that these cells function in relation to a fixed-size module of inner nuclear layer neurones.

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A role for collagen in the pathogenesis of muscular dystrophy?

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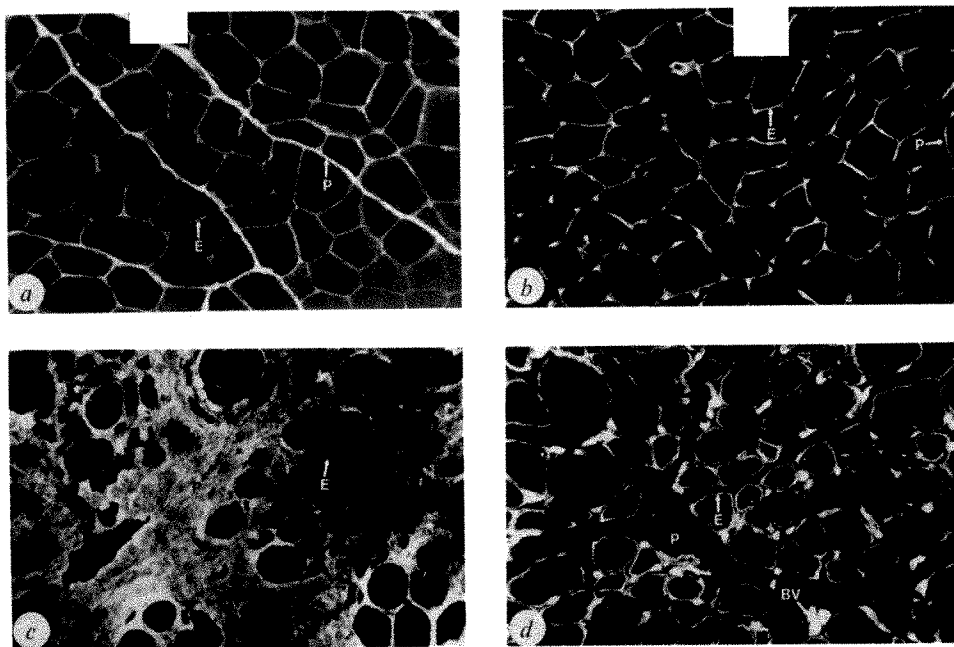
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The extensive proliferation of connective tissue in muscular dystrophy caused Duchenne¹ to term it 'paralysie myosclerosique'. Surprisingly, there has been little interest in the pathogenesis of this marked fibrosis or of the fat replacement in dystrophic muscle. The fibrosis has generally been considered secondary, with various hypotheses² to explain the fundamental cause of muscular dystrophy. Several authors have commented on the increased endomysial stroma early in the disease before any apparent muscle degeneration^{3,4} and have suggested that there might be an aberration in the production of connective tissue in muscular dystrophy and that the thickening pericellular reticulum would adversely affect muscle nutrition^{5,6}. Ionasescu and his collaborators found an over-production of connective tissue with a concomitant decrease in muscle protein synthesis both by polyribosomes from skeletal muscle and by tissue culture of muscle tissue and skin fibroblasts from patients suffering from Duchenne muscular dystrophy^{7–9}. Thomson *et al.*¹⁰ have observed in tissue culture that dissociated muscle from patients with Duchenne and Becker muscular dystrophy will form unusual clusters of 'sticky' cells, which they suggested may reflect an abnormal collagen production. These findings have cast doubts on the interpretation that the extensive connective tissue proliferation characteristic of Duchenne muscular dystrophy, and also seen in limb-girdle, Becker and congenital dystrophies, is simply due to a compensatory replacement of the wasting muscle¹¹ but infers a more primary role for the connective tissue collagen. Only recently has the role of connective tissue in developing muscle come to be appreciated^{12–14}. Moreover, immunofluorescent techniques^{14–16}, now allow investigation of the various types of collagen present in skeletal muscle. We report here on the localisation and change in proportion of collagen types I, III, IV and V in muscle from patients with various forms of neuromuscular disease, and propose a more positive role for collagen.

Needle biopsies were obtained from 15 patients (2–12 yr old) suffering from Duchenne and limb-girdle muscular dystrophy as assessed by clinical, and histological criteria¹¹. Collagen types I,

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Fig. 1 Immunofluorescence staining of transverse sections ($\times 60$) of normal (*a, b*) and dystrophic human muscle (*c, d*) with antibodies to type III (*a, c*) and type IV (*b, d*) collagen. Cryostat sections were stained by the indirect procedure using a fluorescein-labelled anti-rabbit or anti-goat IgG for the second stain. The excessive deposition of type III collagen in muscular dystrophy is readily observed when compared with normal muscle (*a, c*). This increase in type III collagen is distributed in the perimysium (P) and the endomysium (E). The apparent endomysial basement membrane thickening is seen when comparing normal muscle (*b*) with diseased muscle (*d*) stained with anti-type IV antibodies. Note also the more prominent blood vessels (BV) in the diseased muscle. Less dramatic changes are observed with sections stained with antibodies to types I and V (not shown).



III, and IV were localised by fluorescent antibody staining techniques¹⁵ on cryostat sections. The antibodies were raised in rabbits or goats using purified human collagens¹⁷. Antisera were rendered type specific by passage through a series of immunoabsorbent columns with types I–V collagens covalently coupled to Sepharose-4B. The antibodies were assayed by passive haemagglutination for activity and specificity. No cross-reactivity was observed at the lowest dilution used (1/4) with antibody titres ranging over 1/64–1/1,024. The anti-collagen antibodies used for the immunofluorescence staining were diluted to give titres of 1/50–1/100; the fluorescein-conjugated anti-rabbit IgG (Wellcome) and fluorescein-conjugated anti-goat IgG (Miles) were used as recommended by the suppliers.

The distribution of these various collagen types in normal human muscle paralleled that previously found in bovine and chick skeletal muscle^{14–16}. Antibodies to type I collagen stained the epimysium and tendon bundles with slight staining of the perimysium; anti-type III, although staining the epimysium, exhibited a much greater affinity for the perimysium and endomysium between the muscle fibres (Fig. 1*a*); anti-type IV stained the contours of individual muscle fibres intensely, as would be expected for this basement membrane collagen (Fig. 1*b*); anti-type V was also present in the endomysial basement membrane surrounding each muscle fibre, although there was also slight fluorescence in the perimysium.

The most striking feature of Duchenne muscle was the vast anti-type III staining in the peri- and endomysial tissue around the muscle fibres, some of which were small and either atrophying or regenerating and others which were hypertrophied (Fig. 1*c*). The enhanced type III staining was present even in pre-

clinical cases and was a consistent feature of all patients examined. In later stages of the disease the relative increase in type III collagen was proportionately less due to the massive adipose tissue replacement. The vast proliferation of type III collagen in muscle is typical of the response of tissue to injury, as observed in wound healing¹⁸.

The prominent variation in fibre size characteristic of muscle from patients with Duchenne and limb-girdle muscular dystrophies could readily be seen when the endomysial basement membrane anti-type V collagen was stained with anti-type IV (Fig. 1*d*) and anti-type V collagen. Further, the staining of the basement membrane with anti-type IV and V collagen was occasionally more intense around smaller muscle fibres. Whether this reflects a thicker basement membrane or a more extensive infolding of the basement membrane coincident with myofibrillar loss¹⁹ remains to be resolved, perhaps with electron microscopy. Many of the circular profiles which stain prominently with anti-type IV collagen are small capillaries (see Fig. 1*d*). They are much more marked in biopsied muscle in cases of Duchenne muscular dystrophy, which agrees with Jerusalem's observation of reduplicated basement membrane forming two or more continuous or discontinuous layers around the vessel and of an increased mean capillary area²⁰ in Duchenne dystrophy. On the other hand, some of the small fluorescent profiles may be surviving remnants of muscle basement membrane^{21,22}.

The presence of thickened basement membrane and the observation of splits within a few of the muscle fibre bundles from Duchenne and limb-girdle patients are characteristic of muscle disease and muscle injury^{21,23–25}. The appearance of splits in hypertrophied and whorled fibres may denote an early stage in the breakdown of the muscle fibre. Our present finding of type III and IV collagen in these splits (Fig. 2) suggests that collagen may play a part in this process. The recognition of the importance of connective tissue components in muscle disease should provide further impetus in the study of the aetiology of muscular dystrophies.

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Fig. 2 Transverse section of dystrophic muscle ($\times 146$) stained as in Fig. 1 with anti-type IV collagen. Note the presence of staining in the split within the muscle fibre.

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Haloperidol-induced catalepsy is mediated by postsynaptic dopamine receptors

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Antipsychotic or neuroleptic drugs, which block central dopamine receptors, produce a behavioural state in animals in which they fail to correct externally imposed postures. This is referred to as catalepsy. Previous lesion studies have shown that the dopamine receptors in the striatum are involved in this neuroleptic-induced catalepsy^{1–5}. Dopamine receptors, identified by the specific, high-affinity binding of the potent neuroleptic haloperidol, have been shown to be equally distributed postsynaptically on striatal neurones and presynaptically on cortico-striatal terminals^{6–9}. Because the electrolytic lesioning studies^{1–5} have unavoidably damaged both pre- and post-synaptic striatal dopamine receptors, it is not known whether these two receptors are separately involved in neuroleptic-induced catalepsy. Using kainic acid and cortical ablation to destroy postsynaptic and presynaptic dopamine receptors, respectively^{6–9}, the present study demonstrates that the cataleptic effects of haloperidol are apparently mediated by dopamine receptors localised postsynaptically on striatal neurones.

Ten male Wistar hooded rats weighing about 350 g received 6 nmol of kainic acid injected stereotactically into each striatum^{10,11}. Ten control animals received vehicle injections only. In another eight rats the parietal and frontal cortex was removed. Nine control animals received sham operations. All the rats received a prophylactic subcutaneous injection of aquacaine (0.2 ml) and an intraperitoneal (i.p.) injection of diazepam (5 mg per kg) immediately following surgery (diazepam reduces the possibility of extrastriatal damage in rats injected with kainic acid^{12,13}). All animals were allowed to recover for 4 weeks before testing for catalepsy. After testing, the animals were perfused intracardially with 10% formol saline and their brains examined histologically. Consistent with previous reports^{10,11,14,15}, the kainate-lesioned rats showed a considerable loss of neuronal cell bodies in the dorsal two-thirds of the striatum, whereas very little extrastriatal damage was apparent.

In all rats with cortical ablation there was no destruction below the level of the corpus callosum.

The cataleptic effect of the central dopamine receptor blocker haloperidol (1 mg per kg) on kainate-lesioned and cortex-ablated rats is shown in Fig. 1. Catalepsy was measured by gently placing the animal's forepaws on a horizontal bar located 8.0 cm above the floor and timing the period before the animal placed at least one paw on the floor. If an animal remained on the bar for more than 5 min, it was removed and a score of 300 s was given. After saline injections, rats in all groups typically removed their paws from the bar within 20 s. The decorticate rats, however, showed slightly higher descent latencies under saline compared with their control group ($P < 0.05$). This may be due to an increased emotionality, as they defaecated substantially more than controls during testing. Injection of haloperidol induced considerable catalepsy in the control animals (Fig. 1). Haloperidol-induced catalepsy was almost totally blocked in the kainate-lesioned rats ($P < 0.01$). By contrast, haloperidol tended to increase catalepsy in decorticate rats, although this increase did not reach significance ($P < 0.07$).

It has previously been demonstrated^{6–9} that kainic acid-induced striatal lesions destroy about 40% of ³H-haloperidol binding sites in the striatum, demonstrating postsynaptic localisation on intrinsic striatal neurones. Decortication, which removes the cortico-striatal tract, resulted in a similar reduction of ³H-haloperidol binding, showing presynaptic localisation of dopamine receptors on cortico-striatal afferents. Although previous studies^{1–5} have shown that haloperidol-induced

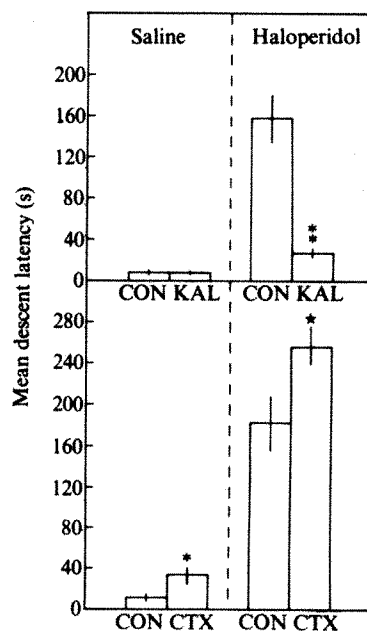


Fig. 1 Catalepsy induced in control (CON), kainate-lesioned striatal (KAL) and cortically ablated (CTX) rats. KAL rats ($n = 10$) were injected with 6 nmol kainate dissolved in 1.0 μ l of phosphate-buffered isotonic saline solution, pH 7.2, as described elsewhere^{10,11}. CON rats ($n = 10$) received infusions of the vehicle. CTX rats ($n = 8$) were anaesthetised with sodium pentobarbital (50 mg per kg i.p.) and positioned on a Kopf stereotaxic instrument. The skin was reflected, the calvaria overlying the parietal and frontal cortex was removed, and the underlying cortex was removed to the level of the corpus callosum by aspiration; bleeding was controlled by gelfoam. CON rats ($n = 9$) received sham operations. The data represent the pooled mean results of four catalepsy tests on each rat carried out at 1-h intervals following drug administration. Haloperidol was dissolved in 0.9% saline and injected i.p. at 1 mg per kg. All animals were injected with either haloperidol or saline in a randomly paired fashion. One week later, the other drug solution was administered. The experiment was run blind insofar as the drug solutions were coded. Control and lesioned groups differed at the following levels of significance using a two-tailed Mann-Whitney U test¹⁶: *, 7%; **, 5%; ***, 1%.

catalepsy seems to be mediated by dopamine receptors in the striatum, the electrolytic lesioning techniques used have been unable to examine specifically the roles of these two dopamine receptors in mediating the behavioural response. My results show that the cataleptic response to haloperidol injection is almost completely abolished in kainic acid-lesioned rats. The marginal increase in catalepsy induced by haloperidol in these animals may be due to the fact that destruction of the striatal interneurons is incomplete. If anything, haloperidol is more effective in inducing catalepsy in decorticate animals. This suggests that haloperidol-induced catalepsy requires the binding of haloperidol to postsynaptic dopamine receptors in the striatum⁶⁻⁹. The present findings may have relevance for understanding differences in antipsychotic potency of neuroleptic drugs in diseases with cortical and/or striatal atrophy.

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Cell-free synthesis of enterotoxin of *E. coli* from a cloned gene

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In a variety of mammalian species including humans the newborn young are particularly susceptible to diarrhoeal disease brought about by toxin-producing strains of the common gut bacterium, *Escherichia coli*¹. Two types of enterotoxin have been described². The first, heat-labile toxin (LT), sharing partial antigenic identity with cholera toxin^{2,3} is comprised of two subunits having molecular weights of 25,500 and 11,500⁴ and, like cholera toxin, stimulates adenyl cyclase activity in the gut⁵. The second type, heat-stable toxin (ST), is a low-molecular weight protein which is excreted into the medium during the growth of ST-elaborating strains. Unlike LT, ST is poorly antigenic and for this reason the identification of ST-producing strains has relied on an assay involving the intragastric injection of infant mice, a test specific for ST⁶. Although many strains produce both ST and LT, strains producing ST alone are especially common in *E. coli* diarrhoeal disease of calves^{7,8}, pigs² and have also been implicated in outbreaks of diarrhoea in humans^{9,10}. Recent evidence suggests that ST activates guanylate cyclase in the gut^{11,12} and its molecular weight has been estimated to be 4,400-5,100 (ref. 13). We have now applied gene cloning techniques to the isolation of ST genes from two porcine *E. coli* isolates and report here the identification of the protein

product of molecular weight 7,000, synthesised in an *in vitro* system directed by the cloned DNA template. This *in vitro* gene product elicits a typical ST enterotoxin response in the infant mouse assay and is most probably synthesised as a 10,000 molecular weight precursor, as might be expected for an exported protein. The gene cloning data also provide evidence that the ST genes and their surrounding transposon structures are probably identical in both porcine and bovine strains of enteropathogenic *E. coli*.

The ability to produce either LT or ST, or both enterotoxins, has been shown in many instances to be a plasmid-mediated trait¹⁴⁻¹⁶. So, Heffron and McCarthy¹⁷ have used molecular cloning techniques to show that the plasmid-borne ST-coding region from a bovine *E. coli* isolate (B41) is located within a transposable DNA element (transposon Tn1681), the structural gene being flanked by inverted repeats of the IS1 insertion sequence.

We have found that commencing with two ST-elaborating porcine isolates (P288 and P310) of *E. coli*, the ST-coding region can be isolated on a *Pst*I endonuclease fragment of molecular weight (MW) 1.05×10^6 , cut symmetrically from within the IS1 sequences of the transposon¹⁸. Since the restriction endonuclease maps of the two porcine *Pst*I fragments are identical in number of sites and size of fragments with the data for the bovine ST gene (ref. 17; M. So, personal communication) we surmise that the ST genes and their transposon structures are identical in all three strains.

The ST-DNA contained within the *Pst*I fragment obtained from the porcine P310 isolate was further subcloned into plasmid pACYC184 (ref. 19). The central *Hae*III fragment was 'flush-end' ligated into plasmid pACYC184 which had been digested with *Eco*RI and the cohesive ends filled in by treatment with avian myoblastosis reverse transcriptase. This results in the regeneration of *Eco*RI sites at the termini of the original *Hae*III fragment. The recombinant plasmid pRIT 10211 so-formed was further manipulated to obtain pRIT 10220 by excising the ST-coding region with *Eco*RI and *Taq*I endonucleases and ligating the resulting fragment into the plasmid vector pBR322 (ref. 20) which had been digested with *Eco*RI and *Cl*aI. In this construction all IS1-sequence DNA has been removed from one side of the ST-DNA insert. The relevant structures of pRIT 10211 and pRIT 10220 are shown in Fig. 1.

These recombinant plasmids were then examined for their ability to stimulate protein synthesis in an *in vitro* transcription-translation system prepared from ST-free *E. coli*²¹. After incubation of plasmid DNA with a reconstituted extract of *E. coli* in the presence of ³H-leucine, newly-synthesised proteins coded by the plasmid were separated by polyacrylamide gel

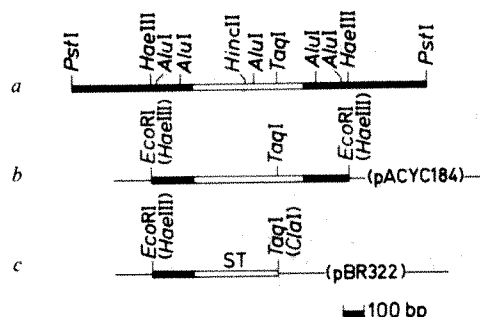


Fig. 1 Restriction endonuclease maps of recombinant ST plasmids. a, Map of the *Pst*I ST-DNA fragment isolated from strain P310 (compare ref. 17). The shaded bars indicate the extent of the IS1 sequence flanking the ST structural gene. b, pRIT 10211: the central *Hae*III ST-DNA fragment has been inserted at the *Eco*RI site of plasmid vector pACYC184. c, pRIT 10220: the *Eco*RI-*Taq*I ST-DNA fragment from pRIT 10211 subcloned into plasmid vector pBR322. The position of the ST gene is inferred from the fact that insertion of a foreign DNA sequence at the *Hinc*II site destroys ST gene activity and that both central *Alu*I fragments are necessary for ST biosynthesis¹⁷.

electrophoresis in the presence of SDS and subsequently revealed by autoradiography. Figure 2a shows that both pRIT 10211 and pRIT 10220 stimulate the synthesis of a protein of molecular weight 10,000 which is absent from the controls.

To identify this protein as the product of the ST gene, an extract of labelled proteins prepared by *in vitro* transcription of pRIT 10220 was analysed for the presence of material reacting with antibody raised against purified ST. Proteins binding non-specifically to antibodies were first removed from the extract by sequential adsorption to pre-immune serum and Sepharose-coupled protein A from *Staphylococcus aureus*. The extract was subsequently incubated with immune serum and the specific antibody-antigen complexes so-formed examined by electrophoresis in the presence of SDS. Figure 2b not only shows that

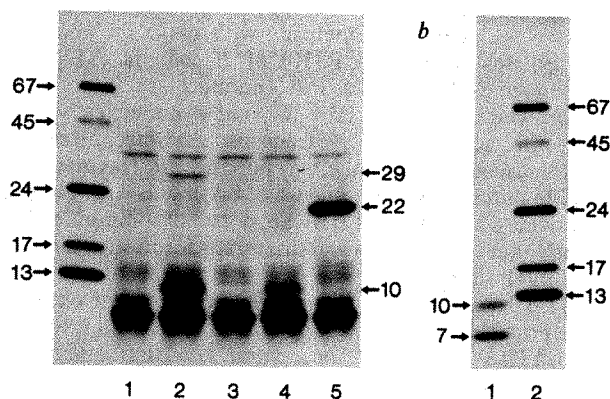


Fig. 2 *In vitro* synthesis and immunoprecipitation of plasmid-coded proteins. *a*, *In vitro* transcription-translation reactions were performed in the conditions described in ref. 21 in a final volume of 30 μ l containing 2 μ g of DNA and 50 μ Ci 3 H-leucine. After 30 min at 37 $^{\circ}$ C the proteins were acid-precipitated, applied to an exponential 8–25% polyacrylamide gel using a standard buffer system²⁴ and subsequently visualised by fluorography²⁵. DNA samples were (1) no DNA, (2) pRIT 10220, (3) vector pBR322, (4) pRIT 10211, (5) vector pACYC184. Figures give the MW $\times 10^3$. The 29,000 MW protein present in slots 2 and 3 has not been rigorously identified; the 22,000 MW protein present in slot 5 but absent from 4 is probably the product of the gene encoding chloramphenicol resistance since the insertion into the *Eco*RI site of pACYC184 disrupts this gene¹⁹. *b*, pRIT 10220 DNA was transcribed and translated as described in (*a*). The sample was diluted to 200 μ l in a final concentration of 0.5 M KCl, 100 mM Tris-HCl pH 8.8, 2% Triton X-100, 100 μ g ml⁻¹ each of soybean trypsin inhibitor and phenylmethyl sulphonyl fluoride, and treated sequentially with 2 μ l of pre-immune serum (1 h, 25 $^{\circ}$ C) and 5 mg protein A-Sepharose (Cl-4B, Pharmacia) (1 h, 25 $^{\circ}$ C). The supernatant after centrifugation was treated with 5 μ l of immune serum (18 h, 4 $^{\circ}$ C) and 8 mg protein A-Sepharose (1 h, 25 $^{\circ}$ C). The Sepharose was collected by centrifugation, washed extensively, and bound proteins denatured and examined by SDS gel electrophoresis and fluorography as before. Slot 1 is the anti-ST immunoprecipitate and slot 2 contains marker proteins. The anti-ST immune serum was raised against modified purified toxin and has been shown to seroneutralise both purified preparations of ST and culture supernatants from ST producing bacteria in the baby mouse test. Preimmune sera from the same rabbits were ineffective in this test.

the 10,000 MW protein reacts with the anti-ST serum, but further reveals a second smaller protein (MW ~7,000) which also reacts with the serum.

The appearance of two proteins both of which react with the anti-ST serum suggests that the larger protein might be the primary translation product of the ST gene, and that the ST protein, in common with many other exported proteins²², would also be subject to specific proteolytic processing associated with trans-membrane transport. In support of this hypothesis are the findings that not only is the *in vitro* transcription/translation

Table 1 Biological activity of ST enterotoxin synthesised *in vivo* and *in vitro*

Source of toxin	No. of mice	Body weight (g)	Gut weight (g)	Ratio
<i>E. coli</i> C600/pRIT 10220 culture supernatant	4	7.415	0.826	0.1214
	4	6.985	0.881	
	4	6.949	0.885	
<i>E. coli</i> C600/pBR322 culture supernatant	4	6.901	0.367	0.0542
	4	6.195	0.351	
	4	6.651	0.352	
<i>In vitro</i> synthesis directed by pRIT 10220 DNA	4	7.023	0.869	0.1237
<i>In vitro</i> synthesis directed by pBR322 DNA	4	6.539	0.418	0.0639
<i>In vitro</i> synthesis mixture, no exogenous DNA	4	6.864	0.427	0.0622

One-day-old mice were injected intragastrically with 0.025 ml of culture supernatant of *E. coli* grown to saturation in Trypticase Soy Broth (Difco) medium or with 0.05 ml of *in vitro* transcription/translation mixture directed by 27 μ g of plasmid pRIT 10220 DNA. The mice were killed after 2 h at 28 $^{\circ}$ C and the carcass and intestines separated and weighed. A gut/body ratio of 0.075 is considered weakly positive, a ratio of 0.100 or above strongly positive.

system partially active in the processing of another exported polypeptide, the penicillinase from *Bacillus licheniformis*²³ but also the published MW of the purified toxin (5,000)¹³ is, within experimental error, in accord with that estimated for the smaller protein (MW 7,000) described here.

By comparison with the relative intensities of the marker proteins in Fig. 2, we were able to estimate that 2 μ g of plasmid pRIT 10220 was capable of directing the synthesis of some 0.5–5 ng of toxin in our standard assay conditions. This result encouraged the scaling up of the reaction and direct biological assay of the products of synthesis by intragastric injection of infant mice. The results shown in Table 1 demonstrate that the incubation of cloned ST-DNA in a coupled RNA- and protein-synthesising system results in the appearance of biologically active heat-stable enterotoxin.

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Vegetative *Dictyostelium* cells containing 17 actin genes express a single major actin

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Although actin is highly conserved between different eukaryotic species¹⁻³, six tissue-specific actins have been characterised in higher vertebrates by complete amino acid sequence analysis (two cytoplasmic actins, two smooth muscle actins and two sarcomeric actins)⁴⁻⁸. Their tissue specificity suggests they may differ in some important although unknown physiological property. Actin expression in lower eukaryotes seems to be a simpler process than in higher eukaryotes since biochemical experiments have indicated only one major type in purified preparations from various species^{1-3,9-12}. However, Firtel *et al.* have isolated several recombinant plasmids containing sequences of *Dictyostelium discoideum* DNA complementary to actin messenger RNA¹³⁻¹⁵ and have suggested that this unicellular slime mould may have 17 actin genes^{15,16} potentially giving rise to several different actins. We have, therefore, determined the complete amino acid sequence of actin from vegetative *Dictyostelium* cells. This sequence is unique and agrees with the DNA sequences of four actin genes for that region of the DNA, which is currently known. The protein sequence does not agree with the three other 'genes' and we discuss the possible expression of minor actin species.

Figure 1 shows the primary structure of actin purified¹¹ from vegetative cells determined by procedures used to study amino acid sequence homologies in actins^{5,6}. Comparison with the sequence of actin from *Physarum polycephalum*³ shows that the two proteins differ by only four exchanges. Three of these involve conservative replacements of uncharged amino acids in positions 189, 228 and 313, and the fourth at position 1 involves a charged residue; here *Dictyostelium* actin has aspartate whereas *Physarum* actin has glutamate. Comparison of the sequences of the two lower eukaryotic actins to those of the mammalian cytoplasmic actins⁶ shows that substitutions are not

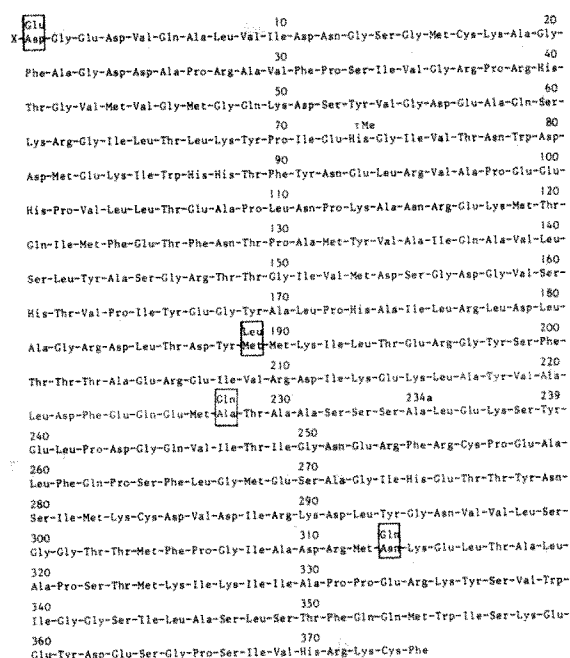


Fig. 1 The amino acid sequence of actin purified from vegetative cells of *Dictyostelium discoideum*. The order of the proteolytic fragments in the polypeptide chain is based on the homology with the known sequence of rabbit skeletal muscle actin¹⁷ taking into account the minor revisions proposed subsequently^{3,6,18}. An extra residue following residue 234 has been found in all actins so far studied and has been most likely overlooked in the original rabbit skeletal muscle actin sequence. To avoid renumbering of the previously documented amino acid exchanges past this residue we follow the proposal¹⁸ to indicate this residue as 234a. X is the acetyl blocking group¹² typical for all actins and His^{Me} is N¹-methyl-histidine. Residues shown in the top line of the boxes indicate the corresponding amino acid residues in the actin from *Physarum polycephalum*³.

distributed randomly, but are concentrated in residues 1 to 6 and occur occasionally past residue 159. Residues 7 to 159 seem conserved, as only one change has been detected. The same region of mammalian skeletal muscle actin differs from cytoplasmic actins by 7 replacements⁶, suggesting perhaps functional importance of this part for non-muscle actins.

Table 1 summarises the codons at the 5'-end of the *Dictyostelium* actin genes which could give rise to amino acid

Table 1 Summary of DNA sequence data for seven actin genes (from ref. 16) compared to the major *D. discoideum* actin (see Fig. 1)

Positions in amino acid sequence	0	1	2	3	12	15	16	17	30	34	38	40
Genes	Codons of DNA sequence of actin genes											
pcDs actin B ₁	AUG	GAC	GGU	GAA	AAU	GGU	AUG	UGU	GUU	AUU	CCA	CAC
pDd actin 2-Sub 1	AUG	GAU	GGU	GAA	AAC	GGU	AUG	UGU	GUU	AUU	CCA	CAU
pDd actin 7	AUG	GAC	GGU	GAA	AAU	GGU	AUG	UGU	GUU	AUU	CCA	CAU
pDd actin 5	AUG	GAC	GGU	GAA	AAC	GGU	AUG	UGU	GUU	AUU	CCA	CAC
Corresponding amino acid in actin of <i>Dictyostelium</i>		Asp	Gly	Glu	Asn	Gly	Met	Cys	Val	Ile	Pro	His
pDd actin 3	AUG	GAA	AGU	GAA	AAU	GGU	AUG	UGU	CUU	AUU	CCU	UAU
M6	AUG	GAC	GGU	GAA	AAU	GGA	AUG	UGU	CUU	AUU	CCU	UAU
									Leu			Tyr
pDd actin 2-Sub 2	AUG	GAA	UGU	GGA	AAA	AGU	AUA	AGU	GUU	AAU	CAA	UAU
		Glu	Cys	Gly	Lys	Ser	Ile	Ser		Asn	Gln	Tyr

Only those codons are given where the presumptive actin gene products give rise to different amino acid residues. Nomenclature for actin genes is taken from ref. 16. The amino acid differences predicted by genes pDd actin 3, M6 and pDd actin 2-Sub 2 are indicated. The methionine residue at position 0 is due to initiation of protein synthesis and removed post-translationally before acetylation of the aspartic acid at position 1 (reviewed in ref. 19).

differences in the N-terminal part of the actin polypeptides (data taken from Firtel *et al.*¹⁶). On comparison with the amino acid sequence (Fig. 1) three different gene types can be distinguished. The first includes pDd actin 2-Sub 1, 5, 7, and pcDd actin B₁, the only clone synthesised from poly(A) containing mRNA. Although these four genes show subtle nucleotide differences they code for the same protein sequence, which agrees fully with the actin sequence given above. In the case of gene pDd actin 7 a complete correlation for the first 67 amino acids is possible. Provided further analysis does not reveal differences between DNA and protein sequence, the genes could all be true actin genes coding, either simultaneously or at different times of the cell and/or differentiation cycle, for the major cellular actin. The second gene type gives rise to actins differing from the major actin in only a few positions; out of the 50 residues currently covered by DNA sequence; pDd 3 actin differs in four and M6 actin in two amino acids (discussed below). Gene pDd actin 2-Sub 2 provides the third gene type. It comprises a putative actin with a high number of exchanges: that is, 10 for the first 40 residues predicted by the current DNA data (Fig. 1, Table 1). Although this divergence (25%) is only slightly higher than that observed in the same region between mammalian skeletal muscle actin and mammalian β -cytoplasmic actin (20%)^{4,6}, a change in the alignment of clustered constant residues typical of the N-terminal regions of all eukaryotic actins^{3,5} so far studied is predicted (note position of cysteine and lysine residues). Therefore if pDd 2-Sub 2 actin is expressed at all, it may be involved in special functions.

Are genes pDd 3 and M6 silent or are they active at a low level or only at specific times during cellular differentiation? Some of the amino acid differences predicted by the DNA sequence would give rise to charge differences in the resulting actins. Several arguments indicate that the two predicted actins could only be expressed at less than 5% of the level of the major actin. First, the tryptic peptide containing at position 30 the presumptive valine to leucine change of M6 and pDd 3 actin (Table 1), would co-migrate in the fingerprint system with the corresponding peptide of the major actin. As the latter peptide does not contain leucine, it is possible to quantify the amount of M6 and pDd 3 actins by the amount of leucine detectable. Amino acid analysis indicates that M6 and pDd 3 actin, if present at all, can maximally account for 5% of the major actin type. Second, the histidine to tyrosine exchange at position 40 (Table 1) would alter the isoelectric point of pDd 3 and M6 actins. This change would also produce an additional more acidic peptide covering residues 40–50. Since such changes were not found, it can be concluded that if genes pDd 3 and M6 are expressed, the corresponding actins can account together for maximally 10% of the final purified actin. Also, because the purification procedure isolates only ~30% of the cellular actin¹¹, the possibility that certain actin forms were lost preferentially cannot be excluded. To avoid this problem we used a method to characterise actins by the identification of the N-terminal tryptic peptide (residues 1–18). This very acidic peptide can be specifically isolated from a tryptic digest of ¹⁴C-carboxymethylated total cellular protein and then characterised in a two-dimensional paper electrophoresis system⁴. Figure 2 shows the *Dictyostelium* actin N-terminal tryptic peptide in this system together with the corresponding peptides from five different bovine actins and *Physarum* actin. The results show that vegetative *Dictyostelium* cells express only one actin (Fig. 2C). Its N-terminal peptide migrates slightly more acidically than the corresponding peptide of *Physarum*, in agreement with the aspartate to glutamate exchange at position 1 (see above). There is no evidence for actin types with N-terminal peptides different from the one predicted by the first type of genes (see Table 1). The N-terminal peptide expected from pDd 3 actin is clearly absent. This peptide should migrate like the *Physarum* peptide as it differs only at position 2 by a serine to glycine change. From the ratios of label present in the spot of the *Dictyostelium* actin peptide and the area in the map corresponding to the position of the presumptive pDd 3 actin derived peptide, the latter actin

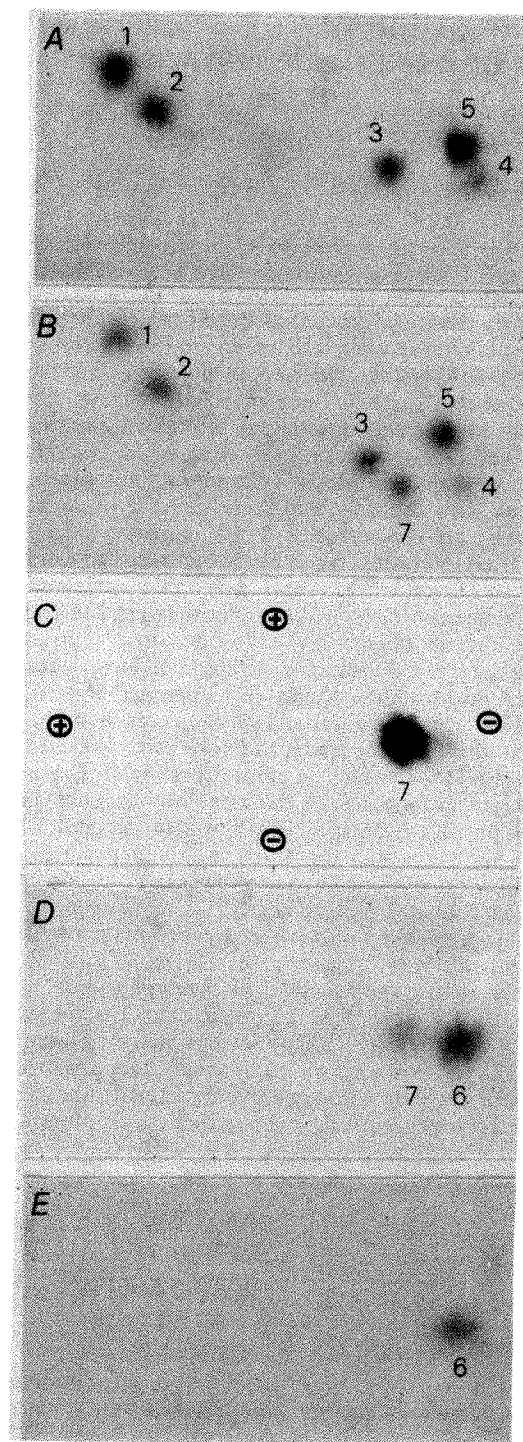


Fig. 2 Two-dimensional separation pattern of the ¹⁴C-carboxymethylated actin N-terminal tryptic peptide from a complete lysate of vegetative cells of *Dictyostelium* (spot 7). This peptide is compared with the corresponding peptides isolated from the following bovine actins: α -like smooth muscle actin of aorta (spot 1), γ -like smooth muscle actin of the rumen (spot 2), β -non-muscle actin (spot 3), γ -non-muscle actin (spot 4), α -skeletal muscle actin (spot 5) and the corresponding peptide from *Physarum polycephalum* (spot 6). A, bovine actins (spots 1–5); B, bovine actins (spots 1–5) + vegetative *Dictyostelium* actin (spot 7); C, *Dictyostelium* actin (spot 7); D, *Dictyostelium* actin (spot 7) + *Physarum* actin (spot 6); E, *Physarum* actin (spot 6). Horizontal separation is by electrophoresis at pH 3.3 and vertical separation is by electrophoresis at pH 6.5. For methods see refs 4 and 5.

could maximally be present at less than 6% of the total actin of vegetative cells.

Thus our studies have not detected minor actin species in addition to the unique actin type for which we provide the complete amino acid sequence. This sequence is compatible with the DNA sequences of four actin genes but does not accommodate the other three currently described. It remains possible that the other genes code for actins present either at very low level in vegetative cells or expressed only at specific times during cellular differentiation, particularly since different isoelectric forms of actin have been reported in one¹⁵, but not a second¹², *in vitro* translation study. The methods described here should provide answers to these questions when cells at different developmental stages and actins translated *in vitro* are used.

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Note added in proof: We have now shown that preaggregation cells of *Dictyostelium* also show the same N-terminal peptide as vegetative cells.

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Long spacers among ribosomal genes of *Drosophila melanogaster*

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The fruit fly, *Drosophila melanogaster*, has 200 tandemly arranged copies of the ribosomal RNA genes (rDNA) per haploid genome^{1,2}. One such cluster of rRNA genes occurs on the X and one on the Y chromosome. The basic repeating unit of the rRNA gene consists of a segment coding for 18S rRNA and 28S rRNA followed by a non-transcribed spacer³ (Fig. 1). In the X chromosome, there are two major size classes (12 and 17 kilobases) and numerous minor size classes of rDNA repeats³⁻⁷. Most of this length heterogeneity is generated by insertions at a specific site in the 28S gene³⁻⁷. The frequency and size patterns of these insertions in the 28S gene differ in the X and Y chromosomes^{4,7,8}. Electron microscopic analysis of rDNA-rRNA hybrids has shown that there is also length heterogeneity in the rDNA non-transcribed spacer^{4,6,7}. This heterogeneity is due in part to internal sequence repetition^{7,9}. We have now examined further the length heterogeneity of the rDNA spacer and have observed a class of spacers that we shall refer to as 'long spacers'. The size and frequency of these long spacers are different in the X and Y rDNA.

The resistance of the rDNA spacer to digestion with the endonuclease *Hae*III (ref. 10) provides a convenient method for the analysis of rDNA spacer size. To determine the spectrum of spacer sizes on the X and Y chromosomes, DNA from X/X or *sc*⁴-*sc*⁸/Y flies was digested with *Hae*III. (*sc*⁴-*sc*⁸ is an X chromosome that is at least 90% deficient in rDNA¹¹.) The resulting fragments were separated by agarose gel electrophoresis and subsequently transferred to nitrocellulose filters by the method of Wahl *et al.*¹², a modification of the Southern technique¹³, which increases the efficiency of transfer of large DNA fragments. The filter-bound fragments were hybridised to ¹²⁵I-labelled spacer DNA¹⁴ and finally visualised by autoradiography. The results of this experiment are shown in Fig. 2. In addition to the expected bands at 4-7 kilobases⁶, fragments at 11, 14 and 20 kilobases are apparent. We shall refer to fragments longer than 9 kilobases as long spacer. To insure that these long spacer fragments were not a result of underdigestion with *Hae*III, the DNAs were redigested with 20-fold excess of enzyme. These experiments yielded patterns identical to those shown in Fig. 2. Digestion with *Hae*III of other samples of X/X and *sc*⁴-*sc*⁸/Y DNAs prepared from independently derived stocks also yielded patterns identical to those in Fig. 2.

To show that the long spacers are continuous with the rRNA structural gene rather than surrounded by other sequences, DNA restricted with *Hae*III was probed with ¹²⁵I-labelled rRNA. In this case, the autoradiographic signal arises from the small segment of 28S rDNA which remains attached to spacer after restriction with *Hae*III (see Fig. 1). Although the intensity of signal of the long spacer fragments was weaker than that observed using ¹²⁵I-labelled spacer DNA as probe, patterns almost identical to those shown in Fig. 2 were obtained. Thus, the pieces of DNA containing the long spacers also contain rDNA genes.

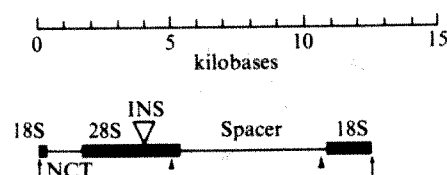


Fig. 1 Repeating unit structure of the ribosomal RNA gene in *Drosophila melanogaster*. The ribosomal gene consists of regions coding for 18S rRNA, 28S rRNA, a non-conserved transcribed (NCT) sequence and a non-transcribed spacer. Length heterogeneity occurs in the insertion (INS) in the 28S rRNA and in spacer sequences. The sites recognised by the restriction enzymes *Eco*RI and *Hae*III are indicated by arrows (↑) and arrowheads (▲), respectively. The map for *Hae*III is incomplete; however, there are no additional sites between the two sites shown¹⁰.

As shown in Fig. 2, digestion with *Hae*III generates different spacer fragment patterns for DNAs derived from X or Y DNA. In addition to the major bands at 5-6 kilobases, there are bands at 20, 11, 7.8 and 7.4 kilobases on DNA from the X chromosome. In DNA from the Y chromosome, bands appear at 14, 11, 5.7 and 5 kilobases and minor bands at 8.9, 7.6 and 6.4 kilobases. Densitometer scans indicate that most (~85%) of the spacer DNA from the X chromosome is 5.7 ± 1.9 kilobases long and that 9.8% makes up the 7.8- and 11-kilobase bands. These results agree with the data of Pellegrini *et al.*⁶, who found ~10% of spacer sequences between 7.5 and 11.5 kilobases and the remainder at 5.67 ± 1.92 kilobases. Their electron microscopic methods, however, would not have detected the 14- and 20-kilobase segments. Approximately 80% of the spacer from the Y chromosome is between 4 and 7.6 kilobases. As there are 200 rRNA genes per nucleolus² and assuming that each is linked to a spacer 5.7 ± 1.9 kilobases long, we estimate from the densitometer scans that there are about three copies of the 20-kilobase spacer fragment and five copies of the 11-kilobase fragment in the X chromosome nucleolus organiser. Similarly,

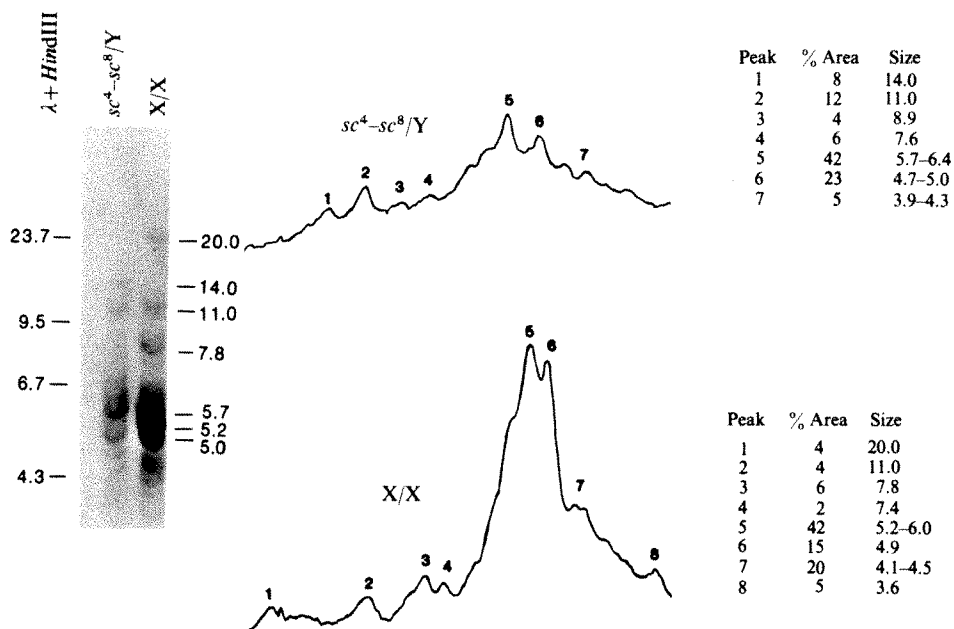


Fig. 2 *Hae*III restriction patterns of rDNA spacer in *Drosophila melanogaster*. DNA (2 µg) from *sc*⁴-*sc*⁸/Y male and ORE-R X/X female flies was digested with *Hae*III, separated on the same 0.7% agarose gel, transferred to a single nitrocellulose filter and hybridised to ¹²⁵I-labelled spacer (specific activity 4 × 10⁷ c.p.m. per µg) DNA and a 200-fold excess of purified unlabelled rRNA. (*sc*⁴-*sc*⁸ designates an X chromosome deficient in at least 90% of its rDNA¹¹.) The size in kilobases of bands from X and Y derived DNA is given on the right. The size of the large fragments was confirmed by electrophoresis through 0.4% agarose. Densitometer scans of the X/X and *sc*⁴-*sc*⁸/Y lanes and the area under each peak are shown on the far right.

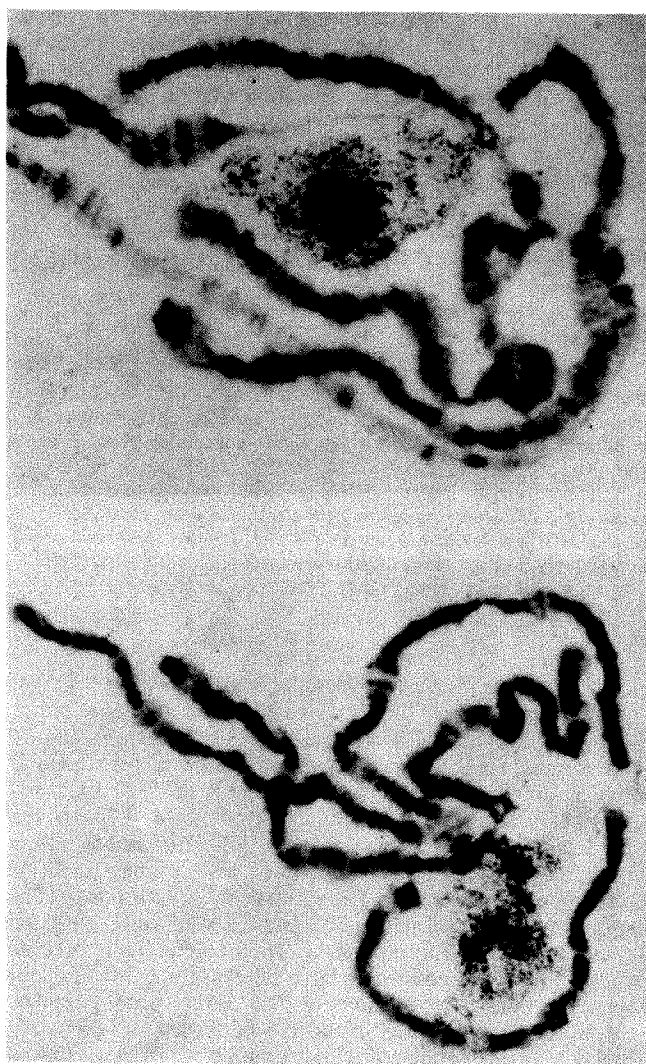


Fig. 3 *In situ* hybridisation of ¹²⁵I-labelled spacer to *D. melanogaster* polytene chromosomes. As noted in these representative nuclei, all grains appear over the nucleolus (exposure, 3 days).

we calculate that there are about 8 copies of the 14-kilobase spacer fragment, and 16 copies of the 11-kilobase fragment in the Y chromosome rDNA cluster.

It is possible that the fragments which hybridise to spacer segments do so because they share homology with the spacer but are not actually part of the structure of rDNA. For example, they might cross-hybridise with centromeric satellite sequences. If so, *in situ* hybridisation of ¹²⁵I-labelled spacer to polytene chromosomes should detect the presence of such sequences elsewhere in the genome. As shown in Fig. 3, the ¹²⁵I-labelled spacer hybridises exclusively to the nucleolus. Even extreme over-exposure (15 d) revealed no grains consistently over any portion of the genome other than the nucleolus. Thus, most, if not all, of the spacer sequences, including the long spacer fragments, are part of the structure of the rRNA genes and are present in the nucleolus.

Electron microscopy and restriction enzyme analysis of cloned and uncloned rDNA repeat units have shown that the non-transcribed spacer is moderately heterogeneous in length (5.7 ± 1.9 kilobases)^{4,6,7} and probably consists mainly of repeated sequence elements^{7,9}. We have shown that spacers longer than 9 kilobases also occur in ribosomal DNA. The organisation of the long spacers has not been further examined and it is possible that in addition to spacer sequences, non-spacer elements such as insertion sequences also reside in these gene units. Although the sequences of the major class of spacers are very similar in the X and Y chromosomes⁷, our results indicate that there are clear size and frequency differences between the long spacers derived from the X and Y chromosomes.

The heterogeneity observed in the size and frequency of long spacer supports the contention of Tartof and Dawid⁸ that the rDNA genes on the X and Y chromosomes are maintained separately without genetic exchange. Two previous pieces of evidence also support this argument. Wellauer *et al.*⁷ and Tartof and Dawid⁸ have shown that type 1 (5 kilobase) inserts which are frequent in X rDNA are absent in Y rDNA, and Yagura *et al.*¹⁵ have recently demonstrated that the primary structure of 18S rRNA transcribed from X rDNA differs from that of Y rDNA. In the light of these observations, it seems unlikely that genetic exchange is responsible for the similarity of transcribed^{7,16} and spacer sequences. Selection pressures may instead have a major role in the parallel evolution of tandemly repeated genes in non-homologous chromosomes.

The presence of a small number of copies of long spacer sequences within the rDNA suggests that the rRNA genes are not organised as a simple tandem array of 200 repeating units. Rather, they may be grouped into subclusters separated from

each other by long spacer. Note that changes in rRNA gene number arising from rDNA magnification or reduction tend to occur as single stepwise increases or decreases of approximately 50 or 100 rRNA genes^{2,17}. On the basis of several experimental lines of evidence, it was initially proposed that rDNA magnification and reduction are driven by unequal sister chromatic exchange¹⁸. It is conceivable that such exchanges may occur at sites in long spacer DNA.

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A *trans*-acting factor mediates inversion of a specific DNA segment in flagellar phase variation of *Salmonella*

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Inversions of specific DNA segments have been shown to be involved in regulating gene expression in several systems, and flagellar phase variation in *Salmonella* is one example. Strains of diphasic *Salmonella* species, such as *S. typhimurium*, possess two non-allelic structural genes, *H1* and *H2*, for flagellin, the component protein of flagellar filaments. One or the other is expressed in a bacterial clone. Lederberg and Iino¹ reported that the switch from the expression of one gene to that of the other was controlled by a genetic element linked to the *H2* gene and that the state of *H2* determined the flagellar phase of the bacterium. Simon *et al.*²⁻⁴ have shown that the expression of *H2* is controlled by a recombination which inverts a region of DNA containing an element necessary for the transcription of *H2*. We report here that prophages P1 and Mu produce a *trans*-acting cytoplasmic factor which mediates the inversion of a specific DNA segment in flagellar phase variation of *Salmonella*.

H1 and *H2* specify phase-1 and phase-2 flagellin, respectively¹. Genetic⁵⁻⁷ and biochemical⁸ studies have shown that *H2* constitutes an operon with *rh1* which specifies a repressor of the expression of *H1*. When the *H2*-operon is active (*H2*-on), phase-2 flagellin is synthesised and *H1* is repressed by the product of *rh1*. When the *H2*-operon is inactive (*H2*-off), *H2* and *rh1* are not transcribed and, thus, phase-1 flagellin is synthesised. The element which determines the state of the *H2*-operon is termed phase determinant (*PD*)⁹. It is adjacent to the *H2*-operon and regulates its activity by inversion²⁻⁴. This invertible segment consists of 800 base pairs of DNA². From genetic studies of a strain of *Salmonella abortusequi*, a species

Table 1 Effect of the exogenous *vh2*⁺ allele on the flagellar phase variation of the *vh2*⁻ strain

Plasmids	Swarm-forming ability on motility agar plates containing anti-flagella antisera			
	none	e, n, x	a	e, n, x + a
None	+	-	+	-
pCR1	+	-	+	-
pKK1200	+	+	+	-

The bacterial strain was KK1027, a *strA recA* derivative of SL23 (Fig. 1). The hybrid plasmid, pKK1200, contained a 3.75-kb DNA fragment of *Salmonella* inserted into the *EcoRI* site of pCR1 (ref. 19). This fragment containing the *vh2*⁺ allele, *PD*, and *H2* but not *rh1* was derived from pJZ200 (ref. 20). This *H2* gene specified e, n, x-flagellin. Plasmids were introduced into KK1027 by F factor-mediated transfer from *E. coli* cells harbouring F' factor and either pCR1 or pKK1200. Motility agar plates contained, per litre of distilled water: 7.5 g tryptone (Difco); 2.5 g NaCl (Nakarai) 2.5 g agar (Shoei). Antisera were prepared from rabbits immunised by flagella. For the motility tests, cells were inoculated on to motility agar plates and incubated at 37°C for 1 or 2 d. +, Motile; -, non-motile.

that changes phase at very low frequency, Iino¹⁰ showed that the *vh2* gene controls the change of state of *H2*, to which it is closely linked. Diphasic strains carry the *vh2*⁺ allele and change their flagellar phase at frequencies of 10⁻³ to 10⁻⁵ per bacterial division¹¹. Stable-phase strains carry the *vh2*⁻ allele and change their flagellar phase at frequencies lower than 10⁻⁷ per bacterial division¹⁰. Thus replacement of *vh2*⁺ by *vh2*⁻ results in stabilisation of the *H2*-operon in whatever state it is at the time of the replacement. A model for flagellar phase variation of *Salmonella* is summarised in Fig. 1.

Among the factors involved in regulating flagellar phase variation of *Salmonella*, *PD* acts *cis* to the *H2*-operon²⁻⁴. To determine whether the *vh2* gene acts in *cis* (and is therefore probably identical with *PD*) or in *trans*, a hybrid plasmid (pKK1200) containing the *vh2*⁺ allele was introduced into the *vh2*⁻ strain (KK1027). This strain was an *strA recA* derivative of the *S. abortusequi* strain SL23 (Fig. 1, ref. 8). It was fixed in phase-2 by the *vh2*⁻ allele and produced flagella antigenically characterised by the antigen e, n, x. The repressed phase-1 antigen type was a. As Table 1 shows, strain KK1027 carrying pKK1200 showed phase variation, producing some sub-clones with antigen e, n, x and others with antigen a. By contrast KK1027 carrying pCR1 or no plasmid failed to change its flagellar phase. The expression of the previously repressed chromosomal *H1* gene specifying antigen a shows that there is no *rh1* product in the cells concerned, and therefore that not only the plasmid-borne but also the chromosomal *H2*-operon is 'off'. Thus the plasmid-borne *vh2*⁺ gene seems to act in *trans*, to

Table 2 Effects of prophage P1 or Mu on flagellar phase variation of *vh2*⁻ strains

Host strains	Plasmids	Production of motile cells
KK1251	None	-
	P1CMclr100	+
KK1252	None	-
	F' lac ⁺	-
	F' lacI ⁺ ::Muc62	+

KK1251 and KK1252 were *galE* and *strA* derivatives of SJW1250 (Fig. 1), respectively. P1CMclr100 was a temperature-inducible mutant of P1 carrying a chloramphenicol-resistance element, Tn9 (ref. 21). Muc62 was a temperature-inducible mutant of Mu-1 (ref. 18). P1CMclr100 lysogens were prepared from KK1251 according to Rosner²¹. (Prophage P1 persists in the plasmid state²².) F' lac⁺ or F' lacI⁺::Muc62 factors were transferred, from *E. coli* strains harbouring either of them, to KK1252 according to Miller²³. For the motility tests, cells were inoculated on to motility agar plates and incubated at 30°C for 5 h. One hundred clones were tested for each experiment. +, Presence of motile cells; -, absence of motile cells.

facilitate the change of state of *PD*, a change that is thought to involve inversion of the segment containing the promoter region of the *H2*-operon. This indicates that the *vh2* gene specifies a cytoplasmic factor that catalyses the inversion of *PD* in the flagellar phase variation of *Salmonella* (Fig. 1).

Bacteriophage Mu DNA contains a 3,000-base pair sequence—the G segment—that can undergo inversion¹², apparently correlated with the formation of infectious phage particles^{13,14}. A similar invertible region has been found in bacteriophage P1 DNA^{15,16} and termed the C region¹⁷. The G segment of Mu445-5 (ref. 18) defective in a *trans*-acting factor for G inversion has been shown to be inverted by the inversion system of P1 (ref. 14). We examined whether the inversion systems of Mu or P1 could invert the *PD* of *Salmonella*.

Derivatives of a *vh2*⁺*H2*[−] strain of *Salmonella* (SJW1250, Fig. 1) were chosen as tester strains. SJW1250 was fixed in phase-2 by the *vh2*[−] allele and had no flagella because of a defect in the *H2* gene. Its repressed *H1* gene was intact and specified flagellin, characterised by antigen *gt*. If this strain changes its flagellar phase from *H2*-on to *H2*-off, *gt*-flagellin will be synthesised and, thus, the bacterium will become motile. Normally, it produces motile sub-clones at a frequency of 10^{−8}. Using this system, we showed that the strains carrying P1 or Mu as prophage produced motile sub-clones at a high frequency.

Table 2 shows that KK1251 (a *gale* derivative of SJW1250) carrying P1Cmclr100 and KK1252 (an *strA* derivative of SJW1250) carrying F'lacI^s::Mucts62 were both able to form spreading colonies (swarms) on motility agar plates. They all produced *gt*-flagella, as revealed by slide agglutination tests with anti-*gt* antiserum. These results indicate that the change of flagellar phase from *H2*-on to *H2*-off occurred in culture. So do they also change flagellar phase from *H2*-off to *H2*-on and manifest phase variation? If their flagellar phase oscillates between *H2*-on (not flagellated) and *H2*-off (flagellated), cultures must contain both motile and non-motile bacteria. Therefore, clones cured of phase must be fixed in whatever state, either motile or non-motile, they are in at the time of curing. Table 3 shows that this was the case. Phages were reintroduced into some of the resulting non-motile clones to exclude the possibility that they were non-motile mutants rather than clones fixed in *H2*-on. The lysogens also changed their flagellar phase from non-motile to motile. Thus flagellar phase changed between *H2*-on and *H2*-off in the *vh2*[−] strains carrying P1Cmclr100 or Mucts62. The rate of phase variation calculated from the results in Table 3 was higher than 10^{−2} per bacterial division in both lysogens. This value was greater than that of *vh2*⁺ strains (for example, 10^{−3} per bacterial division in *S. typhimurium*¹¹). To exclude the possibility that the factor catalysing the inversion of *PD* might be produced by the chloramphenicol-resistance element (Tn9) of P1Cmclr100, temperature-resistant, P1-sensitive, and chloramphenicol-resistant clones were isolated from KK1251 carrying P1Cmclr100. They must have Tn9 inserted in their chromosomes and have been free from phages. The resulting

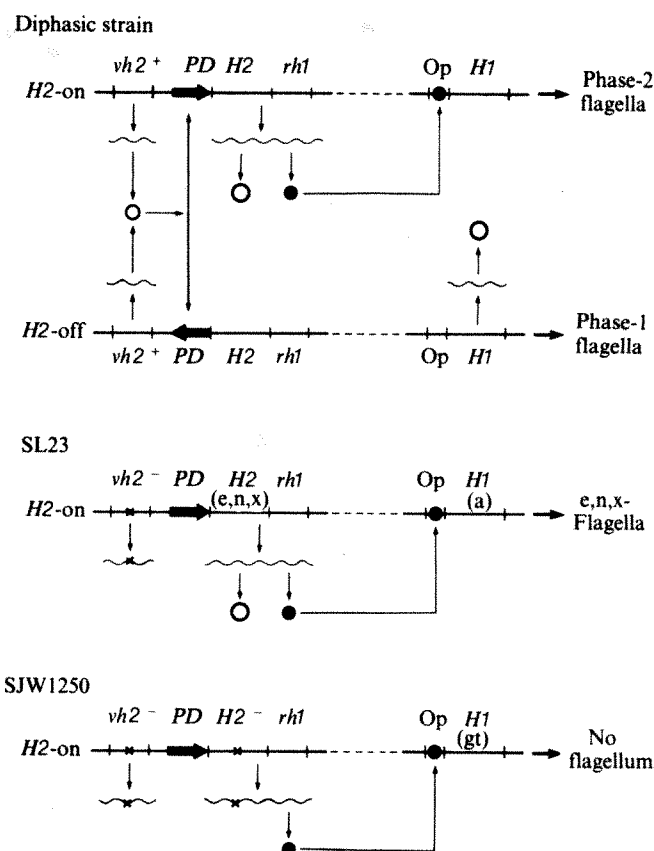


Fig. 1 Regulatory system for flagellar phase variation of *Salmonella*, diphasic strain, SL23 and SJW1250. *H1* and *H2* are structural genes for phase-1 and phase-2 flagellin, respectively. *H2* constitutes an operon with *rh1* which specifies the *H1*-repressor. *Op* indicates the binding site of the *H1*-repressor. *PD* indicates the phase determinant, which regulates the *H2* operon activity through a site-specific inversion. The *vh2* gene specifies a cytoplasmic factor which catalyses the site-specific inversion of *PD*. Horizontal heavy and wavy lines indicate the chromosomes of *Salmonella* and messenger RNA molecules, respectively. Large open and small closed circles represent flagellin and *H1*-repressor molecules, respectively. The small open circle represents the *vh2* gene product. Heavy arrows indicate the orientation of *PD*, which is expressed as the direction of transcription from a promoter residing in *PD*.

individual clones were also fixed in either the non-motile or motile state and did not change their flagellar phase (Table 3). Thus, P1 itself produces the factor catalysing the inversion of *PD*.

It is premature to conclude that the phase-specifying factor which catalyses the inversion of *PD* is also involved in the inversion of G segment in Mu or C region in P1. However, we have cloned the DNA fragment containing G segment of Mu or

Table 3 Flagellar phase of the *vh2*[−] strains cured of prophage P1 or Mu

Host strains	Plasmids	Selected phenotypes	No. of clones tested	No. of clones fixed in		Frequency of phase variation
				motile	non-motile	
KK1251	P1Cmclr100	tr	400	70	330	2.8×10^{-2}
		tr, Cm ^r	200	64	136	2.3×10^{-2}
KK1252	F'lacI ^s ::Mucts62	tr	260	140	120	1.5×10^{-2}

Motile clones harbouring either of the plasmids were grown in LB²³ at 30 °C for about 30 generations. Cultures of KK1251 harbouring P1Cmclr100 were diluted, plated on LB plates or LB plates containing 25 µg of chloramphenicol per ml, and incubated at 42 °C. The resulting isolates on the former plates were tested for P1 sensitivity and chloramphenicol resistance. All were P1-sensitive and chloramphenicol-sensitive and, thus, cured of P1Cmclr100. The resulting isolates on the latter plates were tested for P1 sensitivity, and all of them were found to be P1-sensitive. Thus, they carried Tn9 inserted on the chromosomes but no prophage. Cultures of KK1252 harbouring F'lacI^s::Mucts62 were diluted, plated on MacConkey lactose plates, and incubated at 42 °C. The resulting isolates were all found to form white colonies. For the motility tests, cells were inoculated on to motility agar plates and incubated at 42 °C for 5–24 h. Frequency of phase variation was expressed as the rate of change from motile (*H2*-off) to non-motile (*H2*-on) state per division of cells harbouring either prophage P1 or Mu. tr, Temperature-resistant; Cm^r, chloramphenicol-resistant.

C region of P1 on a plasmid vector, and KK1252 carrying the resulting hybrid plasmids also manifested phase variation (our manuscript in preparation). Thus it is highly plausible that such a factor also catalyses the inversion of G segment in Mu or C region in P1.

We propose that the genes whose products suppress the *vh2*⁻ allele of *Salmonella* should in general be termed *din*. In bacteriophage Mu, a gene which was thought to specify a *trans*-acting factor involved in G inversion was termed *gin*¹⁸. Because the *gin* gene was shown to be near the G segment¹⁸, the *din*⁺ activity of prophage Mu is probably specified by the *gin* gene. Enomoto and Stocker⁹ reported that when the *H2* locus of *Salmonella* together with the *vh2*⁻ allele was introduced into an

Escherichia coli strain by PI-mediated intergeneric transduction, the resulting transductants showed phase variation. This indicates that the *E. coli* strain also has a *din*⁺ activity, though *S. abortusequi* does not.

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Functional organisation of the photo-synthetic apparatus in heterocysts of nitrogen-fixing cyanobacteria

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Many cyanobacteria fix atmospheric N₂ only in specialised cells called heterocysts^{1,2}. Besides containing nitrogenase², heterocysts are distinguished biochemically from vegetative cells by their inability to evolve O₂ or to perform photosystem II activities^{1,3,4} and by a deficiency in the CO₂ fixing enzyme ribulose-1,5-bisphosphate carboxylase⁵. These cells also lack phycobiliproteins and phycobilisomes, that are part of the major light-collecting system for the light reactions of photosynthesis^{1,6}. The phycobilipigments, light-collecting chlorophyll *a* and the photochemical reaction centres constitute a photosynthetic unit (PSU)—the minimum number of pigments necessary to drive photochemistry, oxygen evolution and photosynthetic electron transport. Therefore, the PSU represents an energy generating unit. In cyanobacteria, photosynthesis, and hence the PSU, contributes both ATP and probably a strong reductant to drive nitrogen fixation⁸. Because of the distinct photosynthetic properties of these cells, especially in pigmentation, we have compared the functional organisation of the pigments and photochemical activity of the thylakoid membranes from vegetative and heterocyst cells of *Nostoc muscorum* and *Anabaena cylindrica*. We demonstrate that the PSU is modified during the differentiation of heterocysts to support the specialised energetic requirements of these nitrogen fixing cells.

Axenic cultures of *A. cylindrica* (Cambridge culture collection no. 1403/2a) and *N. muscorum* (strain 7119) were grown in nitrogen-free medium as described previously⁸. Heterocysts and vegetative cells were separated, purified and counted⁸. The purified heterocysts (Fig. 1) were ruptured at 16,000 p.s.i. in a French pressure cell. Membranes were solubilised in Triton X-100 as described¹⁰ and PSU size measured¹¹ as the ratio of total chlorophyll to P700 (the reaction centre of photosystem I). The number of PSUs per cell was calculated from the cellular chlorophyll content and the PSU size¹¹. The spectral properties (300 and 77 K) of whole and detergent-solubilised membranes and fractions thereof were examined in an Aminco DW-2 spectrophotometer. Chlorophyll was determined in 80% acetone using the equations of Mackinney¹²; carotenoids were analysed using the methods of Davies¹³. Polyacrylamide gel electrophoresis of SDS-solubilised membranes was as described¹⁰ and distribution of chlorophyll in the pigmented gel zones was determined from gel scans¹¹.

To characterise the functional organisation of chlorophyll in the PSU of heterocysts and vegetative cells, spectral, photochemical and electrophoretic properties of Triton-solubilised membranes and the photosystem I reaction centre complex, the P700-chlorophyll *a*-protein, isolated therefrom were examined. In Triton-solubilised membranes of vegetative and heterocyst cells of both species, P700 photooxidation and dark reduction kinetics (see Fig. 2) were identical and indistinguishable from those described previously in other cyanobacteria and higher plants¹⁰. However, the wavelength of maximum absorbance decrease of P700 in Triton-solubilised membranes and the P700-chlorophyll *a*-protein occurred at 703 nm in contrast to 697 nm characteristic of P700 in higher plants and many cyanobacteria¹⁴. Previous investigations had demonstrated P700 in heterocysts by chemical difference spectra¹⁵ and by electron paramagnetic resonance spectroscopy¹⁶.

Thylakoid membranes from both cell types and species that were solubilised in SDS and electrophoresed in non-dissociating SDS polyacrylamide gels^{10,17} showed two pigmented zones. The slowest migrating zone was blue-green, stained for protein, and was identical in electrophoretic and spectral properties to the previously described SDS-altered form of the P700-chlorophyll *a*-protein or complex I. The second pigmented zone was green and did not stain for protein. It contained pigment, designated free pigment, that had been released from the native protein by detergent action. Complex I showed the same mobility in SDS gels and the same spectral properties whether isolated from

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Table 1 Distribution of chlorophyll, PSU size and relative numbers of PSU/cell in vegetative and heterocyst cells of *Nostoc* and *Anabaena*

	Heterocysts	Vegetative cells
<i>Nostoc muscorum</i>		
Chlorophyll (μg per cell)	0.7×10^{-7}	1.4×10^{-7}
PSU size (chlorophyll/P700)	60	180
PSU per cell (molecules P700 per cell)	4.0×10^5	2.6×10^5
<i>Anabaena cylindrica</i>		
Chlorophyll (μg per cell)	0.9×10^{-7}	1.7×10^{-7}
PSU size (chlorophyll/P700)	60	180
PSU per cell (molecules P700 per cell)	5.0×10^5	3.1×10^5

Values are the average of at least three experiments.

vegetative or heterocyst cells independent of species. In the vegetative cells, complex I accounted for 20–30% of the total chlorophyll of the thylakoids while in the heterocyst cells it accounted for 70–80%.

Triton-solubilised membranes were used for the isolation of the P700–chlorophyll *a*–protein by hydroxylapatite chromatography^{10,17}. The absorption and photochemical characteristics of this complex isolated from the two cell types and species (Fig. 2) revealed an absorption maximum at 677 nm and a maximum absorbance decrease due to P700 at 703 nm. Only the P700–chlorophyll *a*–protein from *Anabaena* heterocysts showed the lack of a characteristic 490-nm shoulder in its absorption spectrum (Fig. 2). This shoulder has been attributed to β -carotene^{10,19}; indeed, thin layer chromatography of this isolated complex confirmed the absence of carotenoids. The P700–chlorophyll *a*–protein typically contains about 40 ± 10 chlorophylls per P700 in most species thus far examined¹⁴, and pre-

cisely that ratio in both cell types of *A. cylindrica* and *N. muscorum*. Thus, there seems to be no difference between the functional organisation of the centre of photosystem I in the different cells of the two cyanobacteria and that observed in other organisms.

Thylakoid membranes of both species solubilised in Triton X-100 revealed that vegetative cells have a similar chlorophyll to P700 ratio (180 ± 10) to that described previously for other cyanobacteria²⁰. Heterocyst thylakoids were more enriched in P700, exhibiting chlorophyll to P700 ratios of 60 ± 10 (Table 1). As heterocyst PSUs contain essentially no phycobilin pigments or phycobilisomes, the PSU size (ratio of total light-collecting tetrapyrrole chromophores to P700) in these cells is thus only about 60, compared to 300–350 (180 chlorophylls plus 120–180 phycobilin chromophores²⁰) in vegetative cells. Interestingly, the number of antenna molecules per reaction centre in heterocysts is the same small number found in many photosynthetic bacteria²¹. This large reduction in PSU size in heterocysts and the apparent enrichment of P700 is attributed both to the lack of

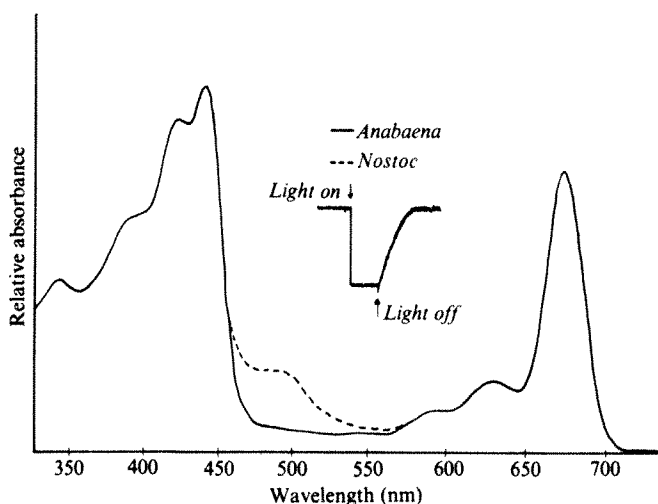


Fig. 2 Absorption spectra of the P700–chlorophyll *a*–proteins isolated from heterocyst thylakoids (at 20°C) of *N. muscorum* (---) and *A. cylindrica* (—). Insert shows kinetics of photooxidation and dark reduction of P700 from *A. cylindrica* heterocyst P700–chlorophyll *a*–protein determined as described previously¹⁰.

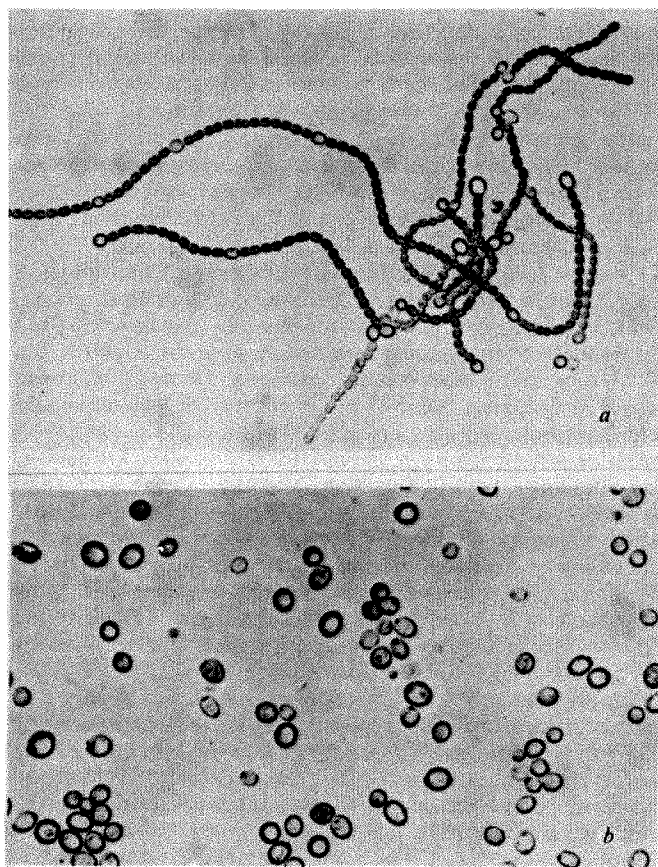


Fig. 1 *Nostoc muscorum*. *a*, Vegetative filaments showing terminal and intercalary heterocysts, the large, thick-walled cells. *b*, Isolated and purified heterocyst cells.

phycobilin pigments, and to a two-thirds reduction in chlorophyll *a*; it closely correlates with the lack of photosystem II (refs 1, 2, 4). Thus much, if not all, of the chlorophyll and all of the phycobilins normally associated with photosystem II in vegetative cells²² is lost during heterocyst differentiation. The loss of detectable photosystem II activity may be related to the lack of phycobilisomes or phycobilin pigments and perhaps of photosystem II chlorophyll–proteins.

In comparison to vegetative cells, *Nostoc* and *Anabaena* heterocysts contain half the chlorophyll content, but have a PSU containing only a third of the number of chlorophyll molecules (Table 1). In both species the number of PSU per cell (Table 1) is 35% greater in heterocysts than in vegetative cells. Therefore, heterocysts show a considerable increase in the cellular content of photosystem I, an ATP and reducing power producing unit. Previous EPR studies¹⁶ also have suggested an enrichment in both P700 and photosystem I bound iron–sulphur proteins in heterocysts. The present study allowed for a direct measurement of the photosystem I content per cell. An increase in photosystem I units should lead to enhanced rates of cyclic photophosphorylation. However, photophosphorylation in heterocysts has been reported to be lower than that found in vegetative cells⁴. Because of the impermeability of isolated heterocysts to reactants, photophosphorylation can be measured only in ruptured cells and this probably contributes to

the lower than expected activity. Hence, until an *in vivo* assay can be developed, it will be difficult to assess photophosphorylation rates in heterocysts accurately.

Several conclusions can be drawn from the present study concerning the relationships between the functional organisation of the PSU and photosynthetic activities, and the N_2 -fixing role of heterocysts. It is clear that heterocysts are capable of supplying sufficient ATP and reducing power to drive N_2 fixation. Although these cells have fairly high rates of oxidative phosphorylation², it has been demonstrated^{4,23} that photosynthesis is the principal energy source of ATP for driving N_2 fixation. Heterocyst differentiation, therefore, should lead to the development of a thylakoid membrane suited for the energetic requirements of the cell. That this is the case follows from several lines of evidence. First, differentiation of heterocysts from vegetative cells results in pigment losses which seem largely restricted to photosystem II. Consequently, photosystem

II activity is eliminated as is production of O_2 , a potent inhibitor of nitrogenase. Second, the pigment alterations lead to the construction of a much smaller PSU; this probably allows for packaging of greater numbers of units within a given cell volume. Third, the reorganisation of the photosynthetic apparatus results in a greatly increased number of photosystem I units which can function to produce ATP and reducing power to support the energetic requirements for N_2 fixation. Finally, the lack of ribulose biphosphate carboxylase eliminates a major competitive system for ATP and reducing power. Hence, the modifications in the photosynthetic systems that occur during heterocyst differentiation seem to be specifically geared to the energetic requirements of these cells.

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Grasses more sensitive to SO_2 pollution in conditions of low irradiance and short days

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Research into the effects of low levels of SO_2 on grasses has revealed no direct correlation between the dose of this pollutant and the plant's response¹. Authors have suggested that environmental factors are of prime importance in the control of the response, because in many cases damage was most severe during the winter^{2,3}. I have investigated the influence of total irradiance on the sensitivity of grasses to SO_2 , and shown that the effect of the pollutant was greatest in conditions of low irradiance and short photoperiod. Together, these factors may contribute to the increased sensitivity of plants during winter exposures to pollutants.

Experiments with a common pasture grass, *Phleum pratense* L. (Timothy), in a wind tunnel fumigation system had shown that plants grown at low irradiance, near the light compensation point, were more sensitive to 11 parts per hundred million (p.p.h.m.) SO_2 than plants grown under higher light levels (T.D., unpublished results). The experiment reported here was a continuation of that work, but irradiance levels and photoperiods representative of winter and summer conditions were used. Early in their growing season, grasses in Britain are exposed to days of about 12 h and low average irradiance, but during mid-summer, days of 16 h and higher levels of irradiance are experienced. Plants were therefore fumigated with SO_2 at these two day lengths, with a low irradiance of 40 W m^{-2} ($125 \mu\text{E m}^{-2} \text{ s}^{-1}$ photosynthetically active radiation, PAR), and higher irradiance of 130 W m^{-2} ($480 \mu\text{E m}^{-2} \text{ s}^{-1}$ PAR). Temperatures were the same for the two treatments, at 20°C (day) and 16°C (night), with a relative humidity of ~60%.

Seven-day old seedlings of Timothy variety S48 were fumigated in 'Perspex' chambers, volume approximately 0.08 m^3 , for

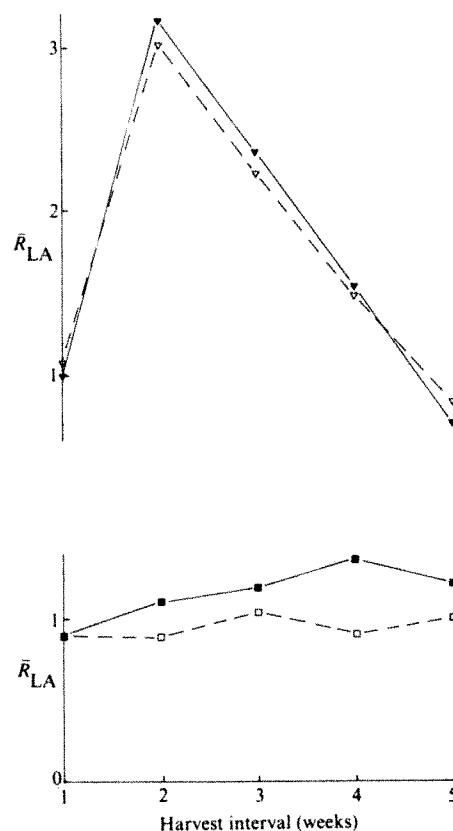


Fig. 1 Mean leaf area relative growth rates (\bar{R}_{LA}) for *Phleum pratense* L. with 12 p.p.h.m. SO_2 (----) or clean air (—). a, High irradiance, long day length; b, low irradiance, short day length.

5 weeks. Each chamber, containing 15 plants each in 7.5-cm pots, received air from a compressor at a rate of one complete air change per minute. In addition, a mixing fan situated inside the chamber rapidly circulated the air over the plants creating a turbulent wind speed of 0.4 m s^{-1} and good pollutant mixing.

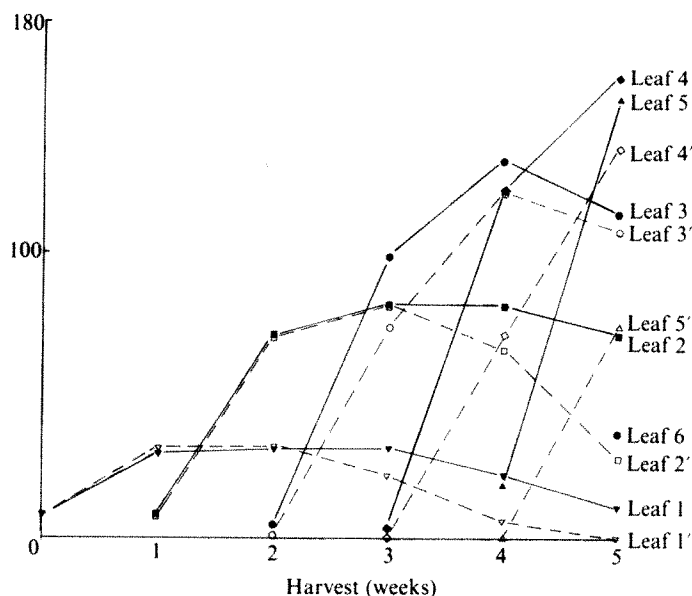
Table 1 Final harvest data giving mean values for *Phleum pratense* L. grown under two light regimes with 12 p.p.h.m. SO₂ or clean air for 5 weeks

	No. of tillers	No. of green leaves	Green leaf area (mm ²)	Green leaf weight (g)	Dead leaf weight (g)	Stem weight (g)	Root weight (g)	Total shoot weight (g)	Total plant weight (g)
High irradiance, long day length									
SO ₂	8.12	26.99	12,291	0.3297	0.0006	0.1768	0.1966	0.507	0.704
Control	8.87	29.24	12,547	0.3384	0.0003	0.1653	0.2212	0.504	0.725
Reduction due to SO ₂	8%	8%	2%	3%	+143%	+7%	11%	+1%	3%
Probability	NS	NS	NS	NS	NS	NS	NS	NS	NS
Low irradiance, short day length									
SO ₂	1.08	3.23	274	0.0099	0.0008	0.0027	0.0015	0.013	0.015
Control	1.54	5.38	744	0.0199	0.0002	0.0060	0.0036	0.026	0.030
Reduction due to SO ₂	30%	40%	63%	50%	+355%	55%	58%	50%	50%
Probability	NS	0.1%	1.0%	0.1%	1.0%	0.1%	1.0%	0.1%	0.1%

NS, not significant.

Two chambers received 12 p.p.h.m. (343 $\mu\text{g m}^{-3}$) SO₂ continuously, and two received clean air containing 0–1 p.p.h.m. SO₂; these acted as the controls. A pollutant concentration of 12 p.p.h.m. is representative of several British urban areas in winter, and does not exceed the highest monthly mean concentrations recorded in Britain⁴.

Each week the plants were removed temporarily and leaf length and width were measured, so that mean relative growth rates of leaf area, \bar{R}_{LA} , throughout the experiment could be calculated (Fig. 1). There was a large difference in relative growth rates between the two light regimes, but the pollutant had no effect in high irradiance and long days. With low irradiance, however, there was a significant reduction ($P < 0.5$) of \bar{R}_{LA} between weeks 1 and 2, and weeks 3 and 4. This early interference in leaf growth could also be demonstrated by recording leaf lengths for the main shoot under low irradiance (Fig. 2). Little difference in leaf growth between SO₂ and control treatments was observed until after the second week of fumigation, when the first and second leaves had almost fully expanded. During the following weeks the senescence of these first two leaves was accelerated greatly by the presence of SO₂. The pollution also reduced the rate of extension of the third, fourth and fifth leaves, and reduced the mean maximum length of the third leaf. At harvest 5 there was no sign of the sixth leaf in the plants treated with SO₂, although in many of the control plants this leaf was quite well developed.

**Fig. 2** Changes in green leaf length of *Phleum pratense* L. with time under low irradiance, short day length, when exposed to 12 p.p.h.m. SO₂ (-----) or clean air (—).

After 5 weeks' fumigation, results of the final harvest clearly demonstrated an effect of SO₂ on plant growth in conditions of low irradiance and short days (Table 1). All shoot and root parameters show ~50% reduction in dry matter accumulation in SO₂, and the green leaf area was particularly affected, for leaf senescence was also promoted. In contrast, SO₂ had no significant effect on the rapidly growing plants grown in conditions of high irradiance and long days. Plants growing in these conditions seem to be particularly resistant to SO₂.

The environmental factors of irradiance and/or day length have therefore had a large effect on the sensitivity of *Timothy* to 12 p.p.h.m. SO₂. Consideration of the biochemical activity of SO₂ within the cell may help to explain this effect. SO₂ readily dissolves on moist cell surfaces to form sulphite ions, SO₃²⁻, and the dissociation products HSO₃⁻ and H₂SO₃, depending on pH. Excess SO₃²⁻ is thought to be toxic to the cell in several ways: it may increase acidity and cause breakdown of chlorophyll *a*, promoting senescence⁵; electron flow within the chloroplasts may be disrupted⁶; SO₃²⁻ may competitively inhibit ribulose biphosphate carboxylase activity with HCO₃⁻, reducing photosynthetic capacity⁷; it has also been suggested that SO₃²⁻ forms addition compounds with sensitive proteins⁸, and that the imbalance caused between partially oxidised and reduced S compounds may inhibit cell division⁹. However, when SO₃²⁻ enters the cell, a large proportion may be oxidised to less toxic SO₄²⁻. This is the usual form in which sulphur is supplied to the plant through the roots, and which is readily assimilated in the chloroplasts. The SO₄²⁻ is metabolised by the sulphate reduction pathway to produce amino acids such as cysteine, methionine and glutathione. This process requires ATP, reduced ferredoxin and amino acid skeletons, all of which would be plentiful in a plant growing in conditions of high illumination and adequate nutrition. However, plants whose growth is severely limited by light will have low reducing power and will not be able rapidly to detoxify SO₂ products along this pathway. Toxic levels of SO₄²⁻ and SO₃²⁻ are, therefore, likely to accumulate and growth may be inhibited.

In the field, plants rarely grow in ideal unstressed conditions. In the experiment reported here, irradiance and/or daylength restrictions have been shown to increase greatly the sensitivity to SO₂ pollution, and other factors of environmental stress are important. Plants, therefore, seem to be more sensitive to SO₂ in the low-growth conditions of the winter, which is also when pollutant concentrations are greatest. Because laboratory studies tend to be conducted in conditions favourable to growth, the impact of atmospheric pollution on vegetation could be much greater than many results have indicated.

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In vivo conversion of a labelled host plant chemical to pheromones of the bark beetle *Ips paraconfusus*

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Chemical constituents of host plants have been suggested to affect in part the ability of certain insects to produce sex pheromones, and thus their ability to reproduce¹. We have investigated such a relationship between the bark beetle *Ips paraconfusus* (Coleoptera, Scolytidae) and its host tree *Pinus ponderosa*. The pheromone system of the insect is well characterised^{2–4} and, as we report here, myrcene, a constituent of the host oleoresin system, is converted in the male beetle to its pheromones, ipsenol and ipsdienol. We used deuterium labelling techniques² to demonstrate for the first time the unequivocal conversion of a host plant chemical to an insect pheromone.

I. paraconfusus males feeding on host phloem tissue produce three terpene alcohols which together are attractive to both sexes—ipsenol (Fig. 1, III), ipsdienol (Fig. 1, II) and *cis*-verbenol. Myrcene (Fig. 1, I) was used because previous studies^{5,6} had indicated that ipsenol and ipsdienol were produced in male but not female beetles when exposed to this terpene.

Myrcene (I) was labelled with deuterium by an unambiguous synthesis at the positions shown to avoid loss of deuterium on conversion to ipsenol or ipsdienol. The oxidation products could be identified by gas chromatography–mass spectroscopy (GC-MS). Myrcene-D was prepared by the method of Westmijze *et al.*⁷. Reaction of 5-chloropent-2-one with methyl-magnesium iodide D₃ produced 5-chlor-2-methyl-pent-2-ol. The alcohol was dehydrated with powdered sodium bisulphite to give 4-methyl-pent-3-enyl chloride (*cis* and *trans* isomers). The latter compound was converted to its cuprate and then added to vinylacetylene to produce myrcene-D. The identity of the products at each step was confirmed by NMR and IR and mass spectral analyses. The NMR of myrcene-D (Perkin Elmer R32, 90 MHz) indicated a deuterium content of approximately 1.6D in the methyl groups at 1.45 and 1.37 p.p.m. No deuterium was observable in any other position. Gas chromatography in conjunction with electron impact and chemical ionisation mass spectroscopy (EI- and CI-GC-MS) confirmed the presence of an isomeric mixture of myrcene-D₃, -D₂, and -D₁ with myrcene-D₂ as the predominant isomer. The loss of deuterium was explained by exchange during the dehydration step.

Ten bark beetles of each sex were sealed in separate 20-ml ampules containing 10 µl of myrcene-D for 18 h as before⁶. Abdomens were then removed and crushed immediately in diethyl ether. Extracts were analysed by electron impact and chemical ionisation GC-MS on 10% Carbowax 20-m column using a Finnigan 4023 system. Peaks corresponding in retention time to ipsenol and ipsdienol, and possessing EI- and CI-GC-MS spectra consistent with the expected fragmentation patterns of ipsenol-D and ipsdienol-D, were observed in extracts of males, but not females. Further analysis by EI- and CI-GC-MS, using a SP-1000 30-m capillary column, confirmed the presence of the two deuterated compounds in male extracts which had coincident retention times with standard ipsenol and ipsdienol. With the exception of the diagnostic pattern of deuterium incorporation, the CI mass spectra of both compounds gave pseudo molecular ions (M+1) and fragmentation patterns similar to ipsenol and ipsdienol. The relative isotopic abundance of deuterium at M+1 for both compounds was also similar to that of deuterated myrcene. Clearly, these compounds were the hydroxylated products of labelled myrcene, deuterated ipsenol and ipsdienol.

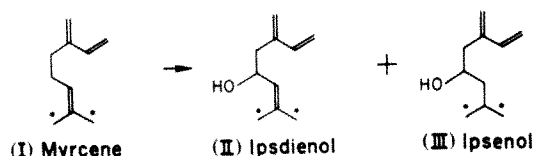


Fig. 1 Structures of myrcene, ipsdienol and ipsenol. Asterisks indicate positions of deuterium.

We conclude that male *I. paraconfusus* can convert myrcene, a terpene hydrocarbon from its host plant, to its pheromones, ipsenol and ipsdienol. Moreover, the presence of other labelled metabolic products in both sexes and the absence of labelled ipsenol and ipsdienol in the female demonstrate the sexual specificity^{2,6} of the biosynthesis of the pheromones. Although we cannot rule out completely alternative pathways (such as *de novo* pheromone biosynthesis, the conversion of other terpene hydrocarbons to pheromones, or induction of pheromone secretion), our results coupled with the observation by Hughes^{5,8} and Byers *et al.*⁶ that ipsenol and ipsdienol are found only in *I. paraconfusus* males exposed to myrcene, support the primacy of host chemicals in pheromone production. If Renwick's demonstration of enantiomeric specificity⁹ in the production of *cis*-verbenol (the non-sex specific component of the pheromone of male *I. paraconfusus*) on exposure to (–)- α -pinene is also a direct biosynthetic step, then all three known pheromone components may be derived from the host plant by simple oxidation of available chemicals. Experiments with isotopically labelled (–)- α -pinene may provide support for this premise. Thus, the evolution of host plant preferences in bark beetles may be a consequence in part of the chemistry of the tree as it affects pheromone biosynthesis.

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Association of lysophosphatidylcholine with fatty acids in aqueous phase to form bilayers

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Lysophospholipids have been implicated in a variety of physiological processes¹. *In vivo* lysophospholipids are invariably produced with fatty acids as the product of hydrolysis by phospholipase A₂ (ref. 2). Therefore, it is of considerable interest to examine the properties of the mixture of these lipids in aqueous suspensions. Results presented here demonstrate that a mixture of fatty acid and lysophosphatidylcholine forms a bilayer type of organisation even though the individual components form micelles when dispersed in an aqueous phase.

1-Acyl-lysophosphatidylcholines were prepared by the action of phospholipase A₂ on phosphatidylcholines in moist diethyl ether. Dispersions of appropriate lipid mixtures were prepared by a standard procedure that is used for the preparation of phospholipid liposomes³ and described in Fig. 1. Freeze-fracture electron microscopy was carried out according to well established techniques⁴. Glycerol was added to the samples to prevent freeze damage.

The aqueous dispersions of lysophosphatidylcholines with various fatty acids are cloudy. When inspected under a polarising microscope they exhibit birefringence, and a flow birefringence can be seen even at less than 10 μ M. Neither of these components dispersed in water alone exhibits such a behaviour^{5,6}. The most distinctive behaviour of mixtures of lysophosphatidylcholine and fatty acid in an aqueous phase is seen in their thermotropic transition profiles. As shown in Fig. 1 the equimolar mixtures of palmitoyl lysophosphatidylcholine with the various fatty acids exhibit a twin endothermic transition at characteristic temperatures that depend on the nature of the fatty acid. Thus, for homologous fatty acids the transition temperature and the enthalpy of transition increase monotonically with the chain length. Other data (not presented here) demonstrate that the transition temperature is lower for the mixtures containing fatty acids with double bonds, such as elaidic and oleic acids. Also, incorporation of cholesterol in equimolar ratios to lysophosphatidylcholine and fatty acids abolishes the transition completely, and substitution of the fatty acid by a long chain alcohol such as *n*-hexadecanol exhibits a twin transition at 327 K (7.45 Kcal mol⁻¹). Such observations demonstrate that the phase transition properties of these mixtures are predominantly governed by acyl chain interactions.

The birefringence and thermotropic phase transition behaviour of lysophosphatidylcholine and fatty acid mixtures suggests the existence of a bilayer-like phase that undergoes a cooperative phase transition. This is confirmed by ³¹P-NMR and freeze-fracture electron microscopy. The ³¹P-NMR spectra over a wide temperature range (one of these spectra is presented in Fig. 2) exhibit asymmetric peaks typical of a bilayer type of lipid organisation⁷. The effective chemical shift anisotropy (CSA_{eff}) is reduced compared with that for phosphatidylcholines. This is not uncommon for lysophosphatidylcholine present in bilayers of other lipids (unpublished observations). Furthermore, the CSA_{eff} is apparently temperature dependent, becoming less at higher temperatures, although a bilayer type of organisation is retained over the 277–327 K range we have examined. As shown in Fig. 3, the freeze-fracture faces obtained on the

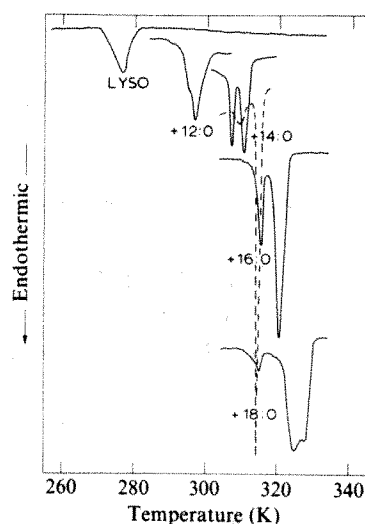


Fig. 1 The DSC scans of equimolar mixtures of palmitoyl lysophosphatidylcholine (LPC) with the fatty acids indicated as label: 12:O-, lauric; 14:O-, myristic; 16:O-, palmitic; 18:O-, stearic. The scans of palmitoyl lysophosphatidylcholine and dipalmitoyl-phosphatidylcholine (broken line) are given for comparison. The experimental details are presented in the text. The transition temperatures and enthalpy for the various mixtures are: LPC (278 K, 3.6 kcal mol⁻¹); DPPC (314.6 K, 8.7 kcal mol⁻¹); LPC+12:O- (297 K, 3.3 kcal mol⁻¹); LPC+14:O- (307 K, 5.0 kcal mol⁻¹); LPC+16:O- (315.3 K, 5.7 kcal mol⁻¹); LPC+18:O- (324 K, 6.7 kcal mol⁻¹); LPC+elaidic acid (302 K, 4.7 kcal mol⁻¹); LPC+oleic acid (290 K, 4.5 kcal mol⁻¹). Appropriate amounts of lipids mixed in chloroform solution were dried under vacuum and then dispersed by vortexing in an aqueous buffer containing 50 mM KCl and 40 mM Tris at pH 8.0 in 25% aqueous ethylene glycol (v/v). Differential scanning calorimetric profiles were determined on 15- μ l samples containing 3 μ mol lipid mixture in sealed aluminium pans on a Perkin-Elmer DSC-2 instrument. The scan range for all runs was 240–340 K, and the scan rate was typically 5 K min⁻¹. Lower scan rates, repeated heating and cooling scans, and dispersions containing no ethylene glycol gave essentially identical results.

dispersions support the existence of vesicular structures of 1,000–6,000 Å diameter. Their fracture planes suggest the presence of bilayer orientation similar to that present in diacyl-phosphatidylcholine dispersions.

The phase properties of palmitoyl lysophosphatidylcholine and fatty acid dispersions in water closely resemble the phase properties of diacylphosphatidylcholines containing similar chains. Such a similarity could result if the acyl chains of lysophosphatidylcholine and fatty acid molecules associate to

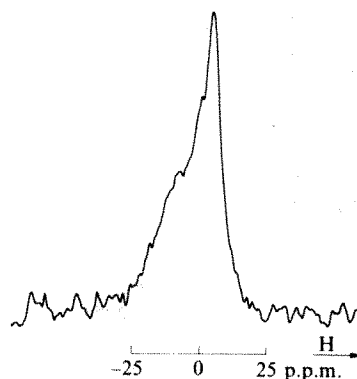


Fig. 2 The 36.4-MHz, high-power proton noise-decoupled ³¹P-NMR spectrum of an equimolar mixture of palmitoyl lysophosphatidylcholine and palmitic acid in 150 mM KCl, 10 mM Tris and 0.2 mM EDTA at pH 7.5 and 314 K. The spectrum was recorded using a spectral width of 12 kHz, 45° radio frequency pulses with pulse rate of 0.17 s and an exponential multiplication resulting in 50 Hz line broadening.

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form a complex that resembles diacylphosphatidylcholine, that is, in bilayers phosphatidylcholine may be considered as a conformationally and orientationally restricted analogue of fatty acid and lysophosphatidylcholine⁹. Although such a concept does not necessarily imply formation of a strong association complex, it is useful in explaining the behaviour of mixed lipid systems^{8,9} as well as the properties of bilayers modified by various solutes³.

A distinct phase behaviour of lysophosphatidylcholine and the fatty acid mixture is also observed in bilayers containing diacylphosphatidylcholine. Studies on aqueous dispersions of the ternary mixture containing equimolar lysophosphatidylcholine and fatty acid with varying mole fractions of diacylphosphatidylcholine (data not presented here) exhibit phase separation over a wide concentration range. Once again the behaviour of the mixture is quite distinct from that of diacylphosphatidylcholine containing either lysophosphatidylcholine or fatty acid⁶.

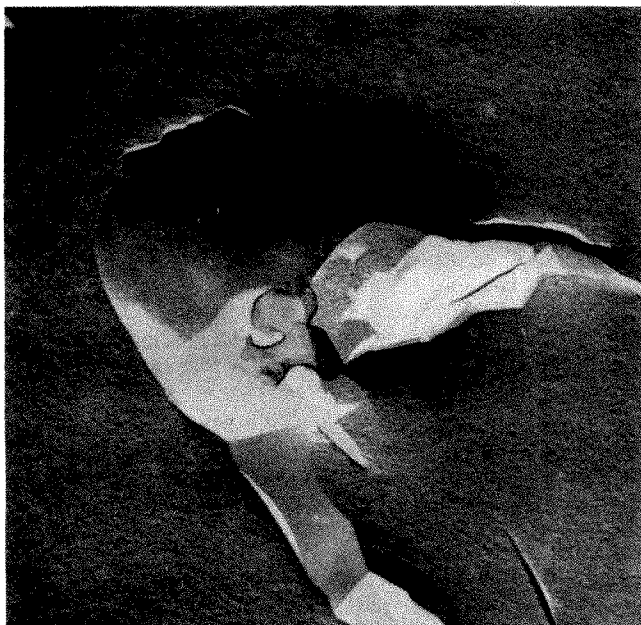


Fig. 3 Electron micrograph of the freeze-fracture replica of dispersions of palmitoylphosphatidylcholine with palmitic acid (1:1) $\times 50,000$.

The full implications of a distinct phase behaviour of fatty acid and lysophosphatidylcholine mixture are under investigation. However, the observations reported here do provide for the first time a physical basis for several yet unexplained observations: certain cell types and organelles have considerable amounts of lytic lysophospholipids but remain viable¹⁰. This could be due to the presence of equimolar proportions of fatty acids as demonstrated for adrenal chromaffin granules (R. Franson *et al.*, in preparation). The latency period during the action of phospholipase A₂ on bilayers is abolished in the presence of both the products of hydrolysis but not by either one of the products alone (unpublished observation). A distinctive behaviour of such ternary mixtures is probably best manifested by the bilayers and biomembranes treated with phospholipase A₂. For example, almost all the phospholipid in the outer monolayer of a bilayer can be hydrolysed with phospholipase A₂ without disrupting the vesicular structure¹¹; the red cells treated with phospholipase A₂ lyse only when they are treated with bovine serum albumin¹², which presumably extracts fatty acids from the membrane, and ³¹P-NMR spectra of phospholipase A₂-treated erythrocyte membranes exhibit retention of bilayer organisation¹³. Such observations suggest that lysophosphatidylcholine and fatty acids together retard haemolysis. Indeed, in the presence of fatty acids the time for lysophosphatidylcholine-

induced lysis is prolonged several hundredfold, and unsaturated fatty acids are more effective than the saturated fatty acids.

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Counting integral numbers of amino acid residues per polypeptide chain

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Proteins have integral numbers of each of the 20 amino acids. However, all the currently accepted methods of determining this number measure the ratio of moles of amino acid residue per mole of protein. This value is rarely close to an integer, due to experimental errors in determination of the molar amounts of both amino acid residues and polypeptide chain¹⁻³. A simple method which gives integral values of amino acid residues per polypeptide chain, independent of any other properties of the protein, would be useful in characterising proteins. We describe here one method; it is illustrated for the case of Cys residues, although the approach should be useful for many of the other 19 usual amino acids.

The method relies on the charge differences introduced by specific chemical modification of the amino acid. In Cys residues this is readily accomplished by reaction of the thiol group with iodoacetic acid, which introduces acidic carboxymethyl groups. Because of the chemical reactivity of thiol groups, Cys residues not reacted with iodoacetate are blocked with iodoacetamide, a similar, but uncharged, reagent.

A complete spectrum of protein molecules having 0, 1, 2, ..., n acidic carboxymethyl groups, where n is the integral number of Cys residues per protein molecule, is generated by reacting all the thiol groups with varying ratios of iodoacetamide to iodoacetate, using competition between the neutral and acidic reagents. To make all thiol groups of the protein chemically equivalent, the reaction is carried out in denaturing conditions, in this case, in 8 M urea.

The molecules with varying numbers of acidic groups are separated by an appropriate method; here by electrophoresis in conditions where the electrophoretic mobility is determined by the net charge of the protein. The number of species ($n + 1$), and hence the number of Cys residues (n), is determined by counting the number of bands.

The procedure is shown in Fig. 1a with bovine pancreatic trypsin inhibitor (BPTI), a small protein known to have six Cys

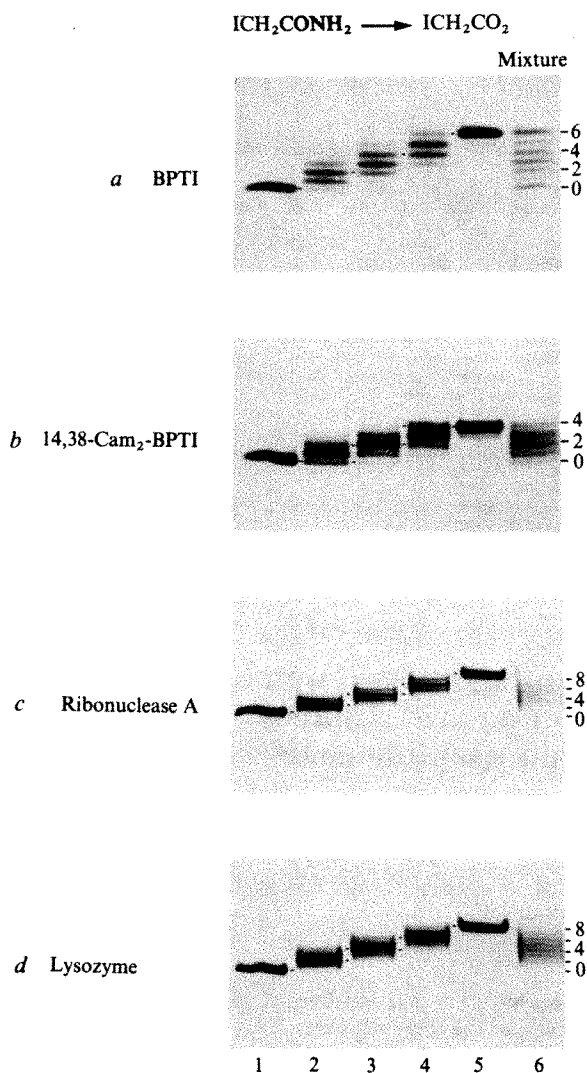


Fig. 1 Electrophoretic separation of protein molecules with 0 to n acidic carboxymethyl groups, where n is the number of cysteine residues in the protein. The reduced proteins were reacted with the neutral iodoacetamide (channel 1, on left), acidic iodoacetate (channel 5), and 1:1, 1:3, and 1:9 ratios of neutral to acidic reagents in channels 2, 3 and 4, respectively. Channel 6 (right) contains a mixture of equal portions of the samples applied to channels 1–5 in *a*, and of channels 2–4 in *b*, *c* and *d*. The bands are counted on the right by the number of acidic groups, and even-numbered bands are connected by dashes between them. The proteins (0.2 mg) were reduced and unfolded by incubation at 37 °C for 30 min in 1.0 ml 8 M urea, 10 mM dithiothreitol, 10 mM Tris-HCl, 1 mM EDTA, at pH 8.0. They were then alkylated by adding 200 μ l of this solution to 50 μ l of 0.25 M iodoacetamide, iodoacetate (adjusted to pH 8.0 with KOH), or mixtures of the two; after 15 min at room temperature, the solutions were placed on ice. Portions of 50 μ l were placed on polyacrylamide gels containing 8 M urea, using the low-pH discontinuous system of Reisfield *et al.*¹⁷; electrophoretic separation was at constant current (100–160 V) at 3 °C for 3–4 h. Staining was with 0.1% (w/v) Coomassie brilliant blue in 10% (w/v) trichloroacetic acid and 10% (w/v) sulphosalicylic acid overnight. The gels were destained by diffusion into 7.5% acetic acid and 5% methanol.

residues^{4–6}. The reduced protein in which the six thiol groups were blocked with iodoacetate migrates electrophoretically more slowly than that blocked with iodoacetamide, due to the six acidic carboxymethyl groups⁷. Reaction of the six thiols with mixtures of the neutral and acidic reagents in three different ratios generated the seven bands expected with six cysteine residues. Approximately equal competition between the two reagents was observed with a ratio of iodoacetate to iodoacetamide of 3:1, indicating that the latter reacts three

times as readily as the former, as has also been observed by others⁸. This mixture produced primarily molecules with 2, 3 and 4 carboxymethyl groups, so the entire spectrum was generated with threefold lower and higher ratios of reagents to generate significant amounts of molecules with 0, 1, 5 and 6 acidic groups. Molecules with 0 and 6 acidic groups were observed when the protein was reacted with just iodoacetamide and iodoacetate, respectively. All seven bands could be demonstrated simultaneously by making a mixture of the above populations.

BPTI, in which two Cys residues (residues 14 and 38) had been blocked covalently by iodoacetamide before competitive modification as described above, so that only four Cys residues were present^{9–11}, gave the expected five bands (Fig. 1*b*). Reduced bovine pancreatic ribonuclease and hen egg lysozyme, each with eight Cys residues^{12,13}, gave the expected nine bands in both instances (Fig. 1*c*, *d*). Bovine α -lactalbumin and β -lactoglobulin clearly demonstrated the presence of eight and five Cys residues, respectively, using an electrophoretic system for acidic proteins. Proteins with no Cys residues, such as the penicillinase of *Staphylococcus aureus*¹⁴, gave only a single band in all instances, with the same mobility when treated with either iodoacetamide or iodoacetate.

The procedure developed here for Cys residues is simple, rapid and gives integral values of the number of cysteine residues per polypeptide chain, independent of any other information about the protein, including its molecular weight. The procedure should be useful in determining the number of Cys residues of other proteins. It requires only a nearly homogeneous preparation of protein (which is also necessary, although not sufficient, for the determination of a correct value using currently accepted procedures) and a method of separating molecules with different numbers of carboxymethyl groups. The separation of species could be enhanced by the use of competition between acidic (iodoacetate) and basic (for example, ethyleneimine)¹⁵ reagents. Other means of separating such species, such as isoelectric focusing or ion-exchange chromatography, may be more appropriate with other proteins.

The general approach should also be useful for counting other amino acid residues for which there are specific reagents to introduce differences in net charge or other properties that may be used to resolve the species. Only a single reagent need be used, and the spectrum of molecules with 0 to n residues may be generated by varying either the time of reaction¹⁶ or the concentration of the reagent. Where two or more different reagents are available, competition between them may be used, as with iodoacetamide and iodoacetate here. The reaction should be carried out in conditions where the protein is unfolded and where all residues of the protein react at similar rates with the reagent. That this was the case with the Cys residues of the proteins studied here¹⁶ is demonstrated by the close agreement of the distributions of molecules with different numbers of carboxymethyl groups with distributions expected to random reaction of each of the Cys residues with the neutral and acidic reagents. The species generated must also be separated in unfolding conditions, where the modified residues have an equivalent effect on the separation procedure. For example, the band of BPTI with one carboxymethyl group per molecule contains molecules in which that acidic group is on any of the six Cys residues of the protein¹⁶, so all six isomeric species must have the same electrophoretic mobility. The other bands must also contain large numbers of species with the carboxymethyl groups distributed over the six Cys residues. Yet the bands are as sharp as those of the homogeneous species with 0 and 6 carboxymethyl groups and are equally spaced.

Any such modification procedure will depend on the selectivity of the reagent and on obtaining stoichiometric reaction of the residues of the pertinent amino acid in limiting conditions, which should be apparent by comparison of the mixtures at varying extents of reaction. Stoichiometric reaction should be indicated by approaching formation of a single species after extensive reaction, as is the case in Fig. 1. Slower reaction at

other sites should be apparent by the slower generation of additional, multiple bands after very extensive reaction.

The procedure developed here will not replace the standard methods of amino acid analysis, as it is unlikely that specific methods will be available for specifically modifying those amino acids with no reactive groups, for example glycine, proline, valine, leucine. Nevertheless, it should be useful for calibrating the molar ratios determined by amino acid analysis, using the integral values determined for one or a few convenient amino acids.

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Brillouin scattering, density and elastic properties of the lens and cornea of the eye

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Brillouin spectra of biological systems may ultimately be related to their intrinsic molecular properties. In some instances the optical properties may be associated with the elastic ones and ultimately with the force constants of the molecules involved^{1,2}. In the present work we have used a triple-pass Fabry-Perot interferometer to measure Brillouin light scattering spectra for refractive tissues of the eye, including cornea, capsule and lens. Combined with corresponding measurements of density, estimates of the real and imaginary parts M' and M'' of the longitudinal bulk modulus have been made for the first time. Measurements have extended over four classes of vertebrate: Mammalia, Aves, Pisces and Amphibia; only small differences have been found between the various samples of cornea, whereas marked differences occur between the different lenses. Hence this account concentrates largely on the latter. The implications of this work lie not so much at the ophthalmological level as at the macromolecular and offer, in conjunction with other scattering techniques, opportunities for probing the lens and its proteins topographically as a function of growth.

The development, morphology, biochemistry, optics and vision of normal vertebrate eyes have been studied for many years³⁻⁷, but comparatively few investigations of the elastic

properties of lenses have been made and none by the method presented here⁸⁻¹⁴.

Brillouin scattering^{15,16} arises when light is scattered by the periodic fluctuations in density that occur in condensed matter as a result of thermally excited hyperfrequency sound waves. The scattered light is shifted in frequency from that of the incident light by typically 1-10 GHz for 90° scattering because of the interaction of the incident light waves with the coherent longitudinal sound waves. The simplest Brillouin spectrum of an isotropic substance therefore consists of the central unshifted Rayleigh line together with two satellites of lower and higher frequency respectively. Aqueous gels of low macromolecular content (~1% by weight) will give Brillouin shifts differing only slightly from that of water, whereas gels of high protein content lead to substantial shifts (ref. 17 and our unpublished data). The high protein content of corneal and lens tissues makes them particularly suitable for the present measurements.

From the differential equation governing the propagation of a longitudinal acoustic wave in a viscoelastic medium the complex longitudinal modulus M^* is given by¹⁸

$$M^* = M' + iM'' = \rho u^2 + i(2\rho u^3 \alpha / \omega) \quad (1)$$

whence

$$M' = \rho u^2 \quad (2)$$

and

$$M'' = 2\rho u^3 \alpha / \omega \quad (3)$$

where ω is the angular frequency of the shift, u the speed of hypersound in the medium α the attenuation defined by the relation

$$\Delta = \alpha u / \pi \quad (4)$$

where Δ is the corrected width of the Brillouin line (FWHM in GHz).

In the arrangement used in these experiments^{2,19} the Brillouin shift Ω is related to the hypersonic speed and the wavelength of incident radiation λ_0 by

$$\Omega = \pm 2n_1 \frac{u}{\lambda_0} \sin \beta \quad (5)$$

when n_1 is the refractive index of air and 2β the scattering angle in air ($90^\circ \pm 1^\circ$).

Provided the density of the sample is known one can therefore determine the longitudinal compressive moduli M' and M'' from measurements of Brillouin scattering data. Characteristic results are summarised in Fig. 1A and B and Table 1.

Collection of data on the lens of the normal human eye has been limited by scarcity of suitable material. The spectra shown in Fig. 1A were taken from one of the two globes so far made available to us and at a time before the development of the density-measuring technique (see legend to Table 1).

The Brillouin shift observed for corneal material, ~4.5 GHz, is lower than that for rat-tail collagen^{1,2}. This is almost certainly due to the fact that the cornea is a complex tissue of collagen, proteoglycans and water. All biological specimens so far examined (including bovine serum albumin, data not shown) show marked increases in Brillouin shift on drying.

Before discussing the data on lenses summarised in Table 1 it is important to note the mode of development through the differentiation of their epithelial cells. Elongated cells are produced at the cortex; and as new layers are formed older ones move inwards. In man and primates this leads to a lens structure of concentric layers of closely apposed fibre cells. In general, the protein content, density and refractive index of the lens increases along any radial line joining periphery to centre. The lens is in broad terms a complex protein gel, the overall protein content of which is about one-third of the lens mass. The chief proteins of the mammalian lens are known as the crystallins, which are globular in character and comprise four predominant classes α , β , γ and δ . Each class contains a number of separable

proteins. Of these the γ class is a closely similar group of cryoprotein monomers of low molecular weight ($\sim 20,000$), while the α and β crystallins (with the exception of β_s) are heteropolymers. The distribution, proportions and molecular weights of the crystallins in the lens change during development as new fibres of different composition are laid down.

Turning to Table 1 and Fig. 1, it is clear that in all lenses studied so far values of density, hypersound speed and elastic modulus (as here defined) all increase from the lens periphery to the centre of the nucleus. This is consistent with Bettelheim and Wang's²⁰ corresponding observations of topographic variation of refractive index in the lens of the calf. In man, cow and sheep there are comparatively small changes of lens density and Brillouin shift between periphery and nucleus. This contrasts sharply with the case of the rat for which a nuclear Brillouin shift of ~ 8.8 GHz has been observed. The quasi-spherical lens of the rat is also substantially different in shape from the generally oval character of those found in the other mammals investigated. The lens of the fowl is rather soft and there is not much difference in elastic modulus between periphery and nucleus. With the bony fishes we come again to lenses almost spherical in shape and with very hard nuclei with appropriately high values of M' . The data for *Sarotherodon mossambicus* (Fig. 1B) and *Perca fluviatilis*

(Table 1) are typical in showing high Brillouin shifts for lens nuclei which are always physically more obvious than in the mammals (other than rodents) and the fowl.

It is interesting to compare the present values of M' of about $3 \times 10^9 \text{ N m}^{-2}$ with the values of Young's modulus for the human lens measured by Fisher^{8,9}. For essentially static distortions values of $3 \times 10^3 \text{ N m}^{-2}$ for the lens and 10^6 N m^{-2} for its isolated capsule were found. We should emphasise that our own values relate to a microscopic scale and that the light scattered is effectively probing high frequency sound wave propagation at wavelengths $< 1 \mu\text{m}$ which is smaller than recorded measurements of lens fibre width. Note that in his lens measurements Fisher takes an average value for lens density, whereas in our work values for small volumes of tissue are measured at positions corresponding to the recorded Brillouin shifts. Our values of longitudinal bulk modulus (M') are of similar magnitude to those found recently for collagen^{1,2}. Kikkawa and Sato¹¹ and Ejiri *et al.*¹² have not given values of the modulus and their results can thus not be compared with the present work.

Fisher and Pettet²⁵ have reported that the lens nucleus in man contains $63.4\% \pm 2.9\%$ water by weight and the cortex $68.8 \pm 4.3\%$. In some animals (Table 1) the nuclear density approaches that of crystalline proteins²⁶ where values of water content by

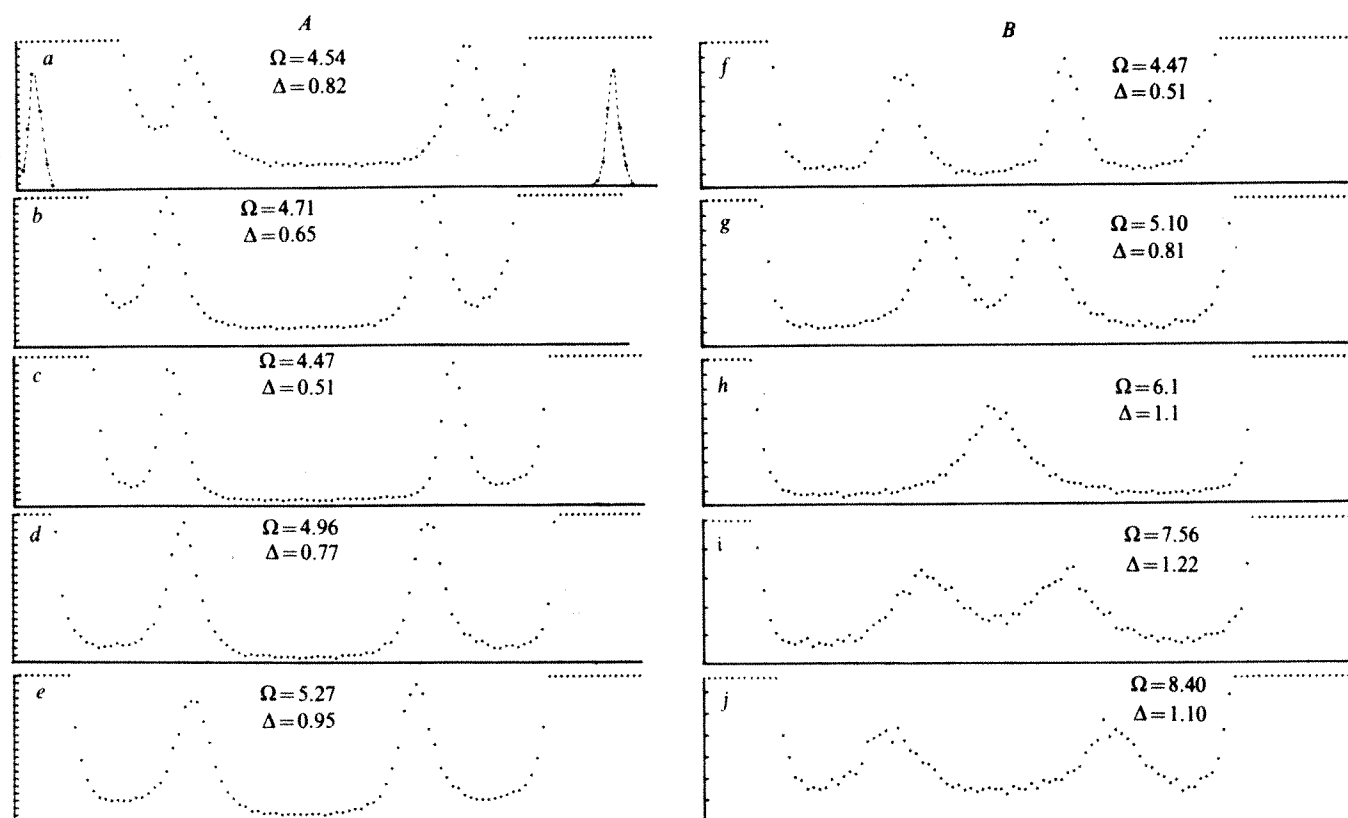


Fig. 1 Eyes were removed from animals within an hour of death and kept in liquid paraffin over ice until the material could be isolated for experiment. In these conditions the cornea and lens tissues showed little spectral change over a period of ~ 2 days. After removal from the globe, material was mounted between microscope slides separated by near-airtight spacers of different thicknesses, but usually $\sim 250 \mu\text{m}$. Samples of cornea were generally thicker than this and with a little Ringer solution gave a good optical seal to the slides and consequently good transmission of the incident laser beam. Two types of lens preparation were employed: either small excised portions $\sim 1 \text{ mm}^3$; or, where spherical as in fish and rat, the whole tissue. The slides were mounted as described previously^{2,19}. With this arrangement the refractive index is not required and the measurements are made at a constant scattering vector \mathbf{K} where $|\mathbf{K}| = (4\pi n_1 \sin \beta) / \lambda_0$ (here equal to $1.82 \times 10^7 \text{ m}^{-1}$). **A**, Brillouin spectra of eye material from man and sheep. The recordings show one complete order with free spectral range 17.0 GHz; instrumental peaks from elastic scattering are superimposed and shown dotted at the top (and greatly reduced). The Brillouin peaks are frequency shifted from their nearest elastic peak: *a*, human cornea (and instrumental peaks); *b*, periphery, human lens; *c*, capsule, sheep lens; *d*, periphery, sheep lens; *e*, nucleus, sheep lens. The lens of the human eye which was examined ~ 4 d post mortem showed a strong yellow fluorescence characteristic of age of subject and its rather cloudy appearance gave very strong elastic scattering. The full scale ordinate is 2,000 counts per channel and the channel width is 173 MHz; the measured shifts and reduced line widths (FWHM) in GHz are shown. **B**, Brillouin spectra recorded at different radial depths in one specimen of the spherical lens of the teleost fish *Sarotherodon mossambicus*. The lens diameter is $\sim 2.0 \text{ mm}$ and the free spectral range 12.2 GHz. The Brillouin shift increases sharply through the series from periphery to nucleus and the peaks in *i* and *j* relate to the further elastic peak. The depths are *f*, 0.06 mm; *g*, 0.28 mm; *h*, 0.52 mm; *i*, 0.76 mm; *j*, 1.0 mm (at lens centre). The measured shifts and reduced line widths (FWHM) in GHz are shown; the intervals on the ordinate scale are 100 counts per channel and the channel width is 122 MHz.

Table 1 Examples of Brillouin scattering, density and elastic moduli for eye tissues of Mammalia, Aves, Pisces and Amphibia

Animal	Tissue	Density ρ (kg m^{-3})	Brillouin shift Ω (GJz) Δ	Spectral width	Hypersound velocity (from equation (2)) u (km s^{-1})	Absorption coefficient $\pi\Delta/u$ ($\times 10^6 \text{ m}^{-1}$)	Longitudinal elastic modulus	
							M' ($=\rho u^2$) ($\times 10^9 \text{ N m}^{-2}$)	M'' ($=2\rho u^3\alpha/\omega$) ($\times 10^6 \text{ Nm}^{-2}$)
Cow (<i>Bos</i> sp.) (1 yr 9 months)	Cornea	1,107	4.43	0.87	1.52	1.8	2.56	5.1
	Lens: periphery	1,105	4.93	0.83	1.70	1.5	3.20	5.3
	Lens: nucleus	1,248	5.36	1.02	1.85	1.7	4.27	8.0
Rat (<i>Rattus</i> sp.) (19 weeks)	Lens: periphery	1,144	5.60	1.09	1.93	1.8	4.26	8.4
	Lens: nucleus	1,358	8.78	1.02	3.03	1.1	12.5	15
Bird Domestic fowl <i>Gallus</i> sp. (30 weeks)	Lens: periphery	1,082	4.61	0.51	1.59	1.0	2.73	3.0
	Lens: nucleus	1,124	4.91	0.61	1.69	1.1	3.21	3.9
Fish (teleost): perch <i>Perca fluviatilis</i> (~7 months)	Lens: periphery	1,103	4.93	0.59	1.70	1.1	3.19	3.8
	Lens: nucleus	1,238	8.75	1.09	3.02	1.1	11.3	14
Amphibian: common frog <i>Rana temporaria</i> (mature)	Lens: periphery	1,108	4.50	0.45	1.55	0.9	2.66	2.6
	Lens: nucleus	1,118	6.6	1.00	2.28	1.4	5.81	9.0

The passively stabilised triple pass interferometer²¹ used in these experiments was essential to bring out the weak Brillouin scattering features observed in the presence of strong elastic scattering at the incident frequency. The light source was an argon ion laser operating in a single mode at 488 nm and at power levels varying between 5 and 20 mw. For most of the material studied the Brillouin scattering intensity was comparable with that of pure water, and spectra with about a thousand counts per channel in each peak could be accumulated in about 10 min. Within the sample, the laser beam was focused to ~30 μm diameter and scattered light was collected from ~25 μm length along the beam, corresponding to a volume of $\sim 1.4 \times 10^{-4} \text{ mm}^3$. The corneas were found to become slightly cloudy within 12 h post mortem and very strong elastic scattering $\sim 10^6$ times the Brillouin scattering intensity was evident. Material from lens and capsule generally showed 1–2 orders less elastic scattering. A gradient density column (originally introduced by Linderström-Lang and Lanz²² as modified by Low and Richards²³) has been used to measure density using a mixture of xylene ($\rho = 865 \text{ kg m}^{-3}$) and chloroform ($\rho = 1,498.4 \text{ kg m}^{-3}$) in a small closed measuring cylinder coated internally with 'Repelcote' to prevent adherence of tissue. The preparation of the column and precautions required followed the lines of the earlier work and will not be elaborated here. Accurate measurements of density can readily be obtained in this way, but it is more difficult to determine precisely the position within the lens from which tissue has been removed and techniques are being developed to overcome this. The values of density given for nuclei are for the nucleus as a whole in rat, fish and frog; in other mammals and birds the specimen was excised from the centre. Attempts to fragment the denser nuclei by cutting slices frequently resulted in complete opacity and it cannot be assumed that the density in this state is normal. The calibration droplets of KBr solutions were checked with a Paar densimeter²⁴.

volume of ~43% occur most frequently and may range from 27 to 65%. In old rat lenses protein concentrations in the nucleus may reach values as high as 0.9 g ml^{-1} corresponding to a water content of only 35% by volume²⁷. The high values of Brillouin shift in certain lens nuclei thus seem to correlate well with high protein concentration and low water content.

Note that in weak gels at low concentrations the Brillouin scattering technique would measure hypersound wave propagation in the solvent, corresponding to $\sim 1,400 \text{ m s}^{-1}$ or $\sim 4.3 \text{ GHz}$. For low concentrations ($c \approx 1\%$) the essential propagation in the gel network is slow; speeds up to 10 m s^{-1} have been found by Brenner *et al.*²⁸ in agarose gels, with elastic moduli varying as $c^{4.1}$ (their equation (4)). A large extrapolation to concentrations of ~35% suggests a speed in the gel lattice of the order of that found for the solvent. This establishes that at even higher protein concentrations as in the lens nucleus the propagation must be essentially dominated by the gel and its structure.

In conclusion we have used Brillouin scattering to determine for the first time the longitudinal high frequency elastic modulus of a substantial variety of vertebrate lenses. This in combination with other scattering techniques summarised below offers an

additional approach to the study of the biophysics of lenses on a molecular scale.

The use of a range of incident light-wave frequencies and scattering vectors as well as variation of temperature would allow the investigation of dispersive effects in lens material and further clarify the differences between the static results of Fisher⁸ and our own dynamic studies. There is also some promise that the use of Raman spectra²⁹ may add precision to the knowledge of protein content and secondary structure in lenses as a function of position and development. X-ray diffraction studies of lens nuclei in particular may help to understand the properties of this high-density material. The use of individual crystallins of the vertebrate eye as model systems is now possible and makes the investigation of the effect of variation of water content on Brillouin spectra of lens proteins an important prospect.

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MATTERS ARISING

A new tectonic model for the central Norwegian Caledonides

HORNE'S¹ recent model for the evolution of the central Norwegian Caledonides requires that the Storen, Hovin and Horg Groups originated as an intra-oceanic arc complex above a westerly dipping subduction zone. It is difficult to reconcile present knowledge of the volcanic and sedimentary rocks of these groups with this hypothesis. The Storen Group metavolcanics are well established as being ocean floor basalts² obducted onto the Baltic Shield in pre-Arenig times³. In the Løkken-Meldal region a younger complex of basic volcanics of late-Arenig age occurs within the Lower Hovin Group⁴. These are considered to have originated in a back-arc marginal basin situation⁵. Calc-alkali volcanics of similar age occur some 100 km further west³. The Lower Hovin Group locally contains meta-andesites, but these are volumetrically less important than the rhyolitic volcanics which predominate in the upper Lower Hovin, Upper Hovin and Horg Groups^{4,6,7} (mid-Ordovician to early Silurian). In the Løkken-Meldal region the volcanoclastic sandstones of the Lower Hovin Group have a quartz content that is considerably higher⁴ than that considered typical of an island arc environment⁸. The sandstones and shales of the Upper Hovin Group have a chemistry and mineralogy consistent with a rhyolitic/granitic (continental?) provenance (unpublished data). The Horg Group contains quartzites and vein quartz pebble conglomerates^{6,7}. Thus neither the volcanic nor the sedimentary rocks of the Storen, Hovin and Horg Groups are typical of those expected in an ensimatic arc complex. Indeed the presence of considerable rhyolitic volcanic products is

often considered to imply an Andean type margin^{9,10}. Also the distribution of late-Arenig vulcanism suggests an arc to the west with a marginal basin opening to the east—this requires subduction of the opposite polarity to that proposed by Horne¹.

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HORNE REPLIES—Ryan has raised some important and difficult problems of provenance and petrogenesis that persist in other parts of the Appalachian-Caledonian orogen as well as in the Trondheim region. I look forward to evaluating the unpublished papers he cites as evidence, particularly those pertaining to the age of obduction and the polarity of subduction.

Many of Ryan's arguments are based on the composition of extrusive and detrital rocks in the Løkken-Meldal area some 60 km west of Selbusjøen. Although I have not studied those rocks I am reluctant to accept generalisations based on petrotextonic pigeonholing when it is well established that volcanics in modern arcs and even some spreading ridges are so variable in composition^{1,2}. The compositions of the volcanic and sedimentary strata that Ryan describes from the Løkken-Meldal area are similar to those of strata of comparable age exposed in the

Bronson Hill anticlinorium of New England^{3,4}. Rhyolites are common in the Ordovician volcanic succession, and they are thought to have been the original core rocks of the Oliverian mantled gneiss domes⁵. Many of the Ordovician and Silurian psammitic strata along the anticlinorium are quartzose, and the early Silurian Clough quartzite that rests unconformably on the volcanics and gneiss domes is conglomeratic with very common pebbles of vein quartz within orthoquartzite matrix⁶. Although there is no easy solution to the provenance problem here or in the Trondheim region, most workers regard the rocks in the Bronson Hill anticlinorium as originating on an ensimatic arc complex within a closing Iapetus Ocean^{7,8}.

The calc-alkaline volcanics in western Norway possibly do represent an arc complex further west than the Trondheim arc. However, the presence of neither a western arc nor ophiolites in the Bergen area⁹ helps resolve the ambiguity of subduction polarity in the Trondheim region. Was there a western Iapetus seaway that closed into a second arc?

GREGORY S. HORNE

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Corrigendum

In the letter '(ADP-ribose)_n participates in DNA excision repair' by B. W. Durkacz *et al.*, *Nature* **283**, 593–596, in Fig. 2 the symbols are incorrectly defined. It should read '△, 0 min; ▲, 40 min; ●, 80 min; and ○, 300 min'. Lines 16–17 of Fig. 2 legend should read 'a, b. The cells were exposed to DMS for 20 min, which was washed out, but 3 mM 3-aminobenzamide was added to the medium for the 300 min recovery period. c, d. The cells were exposed only to DMS'.

Errata

In the letter 'Plasmid-mediated tissue invasiveness in *Yersinia enterocolitica*' by D. L. Zink *et al.*, *Nature* **283**, 224–226, a line was omitted from paragraph 4. Line 4 should read '... Serotype 0:8 strains contained either a plasmid of molecular weight (MW) 35×10^6 , a plasmid of MW 41×10^6 or both (whereas...)'.

In the letter 'Do genealogical patterns in purple photosynthetic bacteria reflect interspecific gene transfer?' by C. R.

Woese *et al.*, *Nature* **283**, 212–214, the x axis in the upper part of Fig. 1 should be labelled S_{AB} and the x axis in the lower part should be labelled % Sequence homology.

In the letter 'The relationship between coding sequences and function in haemoglobin', by W. A. Eaton, *Nature* **284**, 183–185, on p. 184 second paragraph line 11 should read '...(2) Residues in the $\alpha_1\beta_2$ contact'.



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BOOK REVIEWS

Impressive inhibitors

Michael Cannon

THIS book is published by Wiley-Interscience as a sequel to *Nucleoside Antibiotics* which appeared in 1970. It describes the application of naturally occurring nucleoside and nucleotide analogues as biological probes in cellular reactions. The compounds listed and considered inhibit between them an impressive range of events including cell wall synthesis, protein synthesis, DNA and RNA synthesis, purine and pyrimidine interconversions, and the activities of both cyclic-AMP-phosphodiesterase and adenosine deaminase. Several of the compounds are of medical importance since they have limited use in the treatment of cancer and viral infections in humans.

It is of no surprise to find an old friend — puromycin — featured in the text. Many biochemists have successfully experimented with this most useful antibiotic to study the mechanism of peptide bond formation on ribosomes, the release of incomplete nascent polypeptide chains and the properties of the ribosomal binding sites for transfer RNA. What may be surprising to some, however, is the fact that there are 69 other compounds described here of which 40 or so have been discovered since the 1970 volume was published. The number of additional events inhibited has increased dramatically in line with the number of new compounds identified. Medical implications are also clearly indicated. In 1970, for example, only 5-azacytidine, of the nucleoside antibiotics, had been used to treat cancer, showing good activity against certain leukaemias and limited activity on some solid tumours, although predictably the drug frequently produces unpleasant side effects. Of the newer compounds described in the present volume, both the pyrrolopyrimidine nucleoside antibiotic tubercidin and 9- β -D-arabinofuranosyladenine (ara-A), the latter having low myelosuppressive activity in humans, have been cleared by the Federal Drugs Administration for the treatment of human basal cell carcinoma, herpes

Nucleosides as Biological Probes. By R. J. Suhadolnik. Pp.364. (Wiley: New York and Chichester, UK, 1979.) \$50, £23.

simplex keratitis and herpes simplex encephalitis. Tubercidin is also a potent anthelmintic agent, as is the pyrimidine nucleoside disaccharide analogue hikizimycin (anthelmycin).

The presentation in this book is fairly standard for each entry and usually covers discovery, production, isolation, physical properties, chemical and biological properties, structural elucidation, biosynthesis, toxicity and where known the mode(s) of action. The 'interest generation' is extremely variable. Some compounds come over as fascinating and remarkable inhibitors. Thus coformycin and 2'-deoxycoformycin inhibit adenosine deaminase, an important enzyme in purine metabolism, a deficiency of which produces an inborn error of metabolism associated with severe combined immunodeficiency disease. The role of these two compounds as immunosuppressants aids the success of tumour grafts and indicates a usefulness in organ transplantation. Other compounds, by contrast, come over as being somewhat boring but it is fair to say that in each case beauty, or lack thereof, will always be in the eye of the beholder.

All but two of the ten chapters end with a particularly valuable summary section drawing together many of the important facts in concise form. Of the two chapters lacking a summary one deals exclusively, albeit briefly, with clitidine and tells us very little of the biological properties of this pyrimidine nucleoside, although we do discover that it resides in the mushroom *Clitocybe acromelalga* and is a physiologically active substance. One presumes that little is, in fact, known about this compound. The remaining summary-less chapter deals equally briefly with the herbicides A and B, 5'-O-glycosyl-ribonucleosides, raphanatin and 6-benzylamino-7- β -D-glucopyranosylpurine. Curiously we are told that the herbicides

show promise as weed killers but are not toxic to mice (or gardeners?).

The text is profusely illustrated not only with the chemical structures of the various compounds described but also with a large selection of diagrams and tables reproduced from original papers. I can understand the author's wish to underscore his factual writing with real experimental data but I found this aspect of the book very annoying on occasion. Certainly these entries fill out the volume but much of the original experimental data presented are, in my opinion, redundant. For example, I am prepared to accept the author's word that cordycepin (3'-deoxyadenosine) inhibits the synthesis of ribosomal RNA and if I found this observation of particular interest I should naturally read the relevant reference. I see little reason why the present text should labour the point by illustrating an experiment involving agarose gel electrophoresis of ribosomal RNA from L1210 cells treated with the inhibitor. Again, an illustration is hardly necessary to support the statement that whereas RNA polymerases isolated from either *Escherichia coli* or T3 bacteriophage are not affected by α -amanitin, the former enzyme is completely inhibited by the naturally occurring nucleotide analogue thuringiensin whereas the latter enzyme is not. There are many other such examples throughout the text.

Overall, however, this will be a useful volume for those who are interested in the subject area — although whether or not the number of such individuals represents a viable economic proposition for Wiley-Interscience, and hence for Professor Suhadolnik, is debatable. Certainly, though, the text makes the reader aware of the remarkable versatility of nucleoside and nucleotide analogues with respect to their inhibitory effects and allows for interesting speculation in the fascinating field of structure-function relationships.

Michael Cannon is Lecturer in Biochemistry at King's College, London, UK.

Mammalian vestibular physiology

R.H.S. Carpenter

Mammalian Vestibular Physiology. By V. J. Wilson and G. Melvill Jones. (Plenum: New York and London, 1979.) £20.48.

WHY do neurophysiologists choose to study one part of the brain rather than another? Amongst sensory systems, at least, it seems to be those which give us the greatest sensual enjoyment that attract the closest investigation: if we add to this the inevitable band-waggon effect, the result is a strikingly disproportionate amount of effort on vision and to some extent hearing, and rather little on anything else. The vestibular system has fared particularly badly from this: apart from the ambiguous delights of the roller-coaster and sky-diver, the system comes to most people's notice only at times of distinct misery — a bad Channel crossing, the acute effects of alcoholic overindulgence — and its contribution to the unending task of keeping us precariously balanced on two tiny supports goes largely unnoticed and unappreciated.

Of course, it has to be admitted that the vestibular system is somewhat redundant, in the sense that much of the information about head position and movement that it provides is also furnished (though more slowly) by the visual system and by proprioceptors in our joints. A man whose vestibular system has been destroyed by disease may not be aware that much is wrong until these alternative sources of information are also unusable — running over rough ground in the dark, or swimming underwater. But as Wilson and Melvill Jones make abundantly clear, these parallel visual and proprioceptive inputs seem largely to make use of the neural circuitry that was originally evolved to process vestibular information; and the study of central vestibular pathways has shed a good deal of light on how information from the eyes and other receptors is used for the same purpose.

One aspect of this cooperation that is particularly interesting, the importance of which has only recently been fully appreciated, is the way in which the matching of visual and vestibular signals is a function that must be *learned* by the brain: this calibration of one input in terms of the other seems to be going on all the time, ensuring that degenerative and other changes in the performance of the vestibular organs do not result in inappropriate responses. A dramatic way of demonstrating this is to get a subject to wear prisms that reverse his visual field: after only a matter of days it is

found that certain of his purely vestibular responses — ones that can be evoked in the dark — follow suit by reversing their direction as well. These changes are almost certainly the result of functional alterations in cerebellar circuitry, and offer the exciting possibility of studying motor learning by the cerebellum in a simple and quantifiable way.

Mammalian Vestibular Physiology provides a first-class account not only of these more recent areas of interest but also of the classical anatomy and physiology of the vestibular organs and their central connections, and fills much more than adequately what has long been an obvious gap in the literature. It is sufficiently wide

in its scope to be of interest to neurophysiologists in other areas as well as to specialists in vestibular and oculomotor physiology, who will certainly find both its intelligent and impartial reviews of difficult topics, and its notably comprehensive bibliography, of more than ephemeral value. Perhaps it may also help to lure neurophysiologists away from the rather obvious charms of visual physiology to a field which promises to begin to unveil the more challenging mysteries of the motor system. □

R. H. S. Carpenter is Lecturer in Physiology at the University of Cambridge, UK.

Laser fusion

Siegbert Witkowski

The Physics of Laser Fusion. By H. Motz. Pp.290. (Academic: London and New York, 1979.) £17; \$37.

IT is a rather bold undertaking to write a textbook on the physics of laser fusion, for essentially two reasons. Firstly, laser fusion is a very rapidly developing field. The interpretation of the complex phenomena has only reached a quasi-steady state which has not yet been proven to be stable against new experimental results. Secondly, the physics of laser fusion comprises a large variety of quite different topics. Therefore, selection of the proper material for an introduction to this field is a major problem. The author has succeeded in this difficult task. He has chosen the essentials, concentrating on the basic phenomena, which are well established. The physics of the nuclear reactions and of high power laser systems

are only touched on briefly in the introductory chapters in order to acquaint the reader with the problems involved and the state of the art. The main part of the book deals with light plasma interaction processes, the excitation, propagation and interaction of waves in plasmas and their relevance to laser fusion. The complex phenomena connected with energy transport in the overdense region are touched on in a special chapter preceding the treatment of the hydrodynamics and its stability. Besides the analytical treatment of the phenomena, results from computer codes are also presented. Each chapter is followed by a list of references where more detailed information can be found on specific subjects. In conclusion, this book can be recommended as an introduction to the field of laser fusion for newcomers, but it is also a useful compilation of the basic physics of laser fusion for the specialist. □

Siegbert Witkowski is a member of the Board of Directors, Projektgruppe für Laserforschung, Munich, FRG.

Models of *r* and *d*

Alan Grafen

The Natural Selection of Populations and Communities. By D.S. Wilson. Pp.186. (Benjamin Cummings: Menlo Park, California, 1980.) \$12.95.

IN whose interest does an animal or plant behave? Evolutionary theorists believe that the workings of natural selection ensure that organisms act as if they were maximizing their reproduction (or more precisely, a weighted sum of their own and their relatives' reproduction). So although some kinds of behaviour might easily be explained as an individual acting in the interests of some larger unit such as its

group, species or community, the orthodox view is still that only in extreme and implausible conditions can this type of explanation be admitted. In *The Natural Selection of Populations and Communities*, D.S. Wilson attempts to give a theoretical justification for accepting these 'higher' levels of selection.

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IMMUNODEFICIENT ANIMALS IN CANCER RESEARCH

Edited by Stephen Sparrow, Pathologist at the Medical Research Council's Laboratory Animals Centre.

1980; £20.00; 208pp; ISBN 0 333 275500

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This book brings together for the first time a variety of animal models in cancer research, from the simple immunodeficient foetus to the complex genetic manipulation needed to produce the 'Lasat' mouse deficient in both thymus and spleen.

This is the first book to deal with this aspect of cancer research in such detail and it will be valuable reading for all those involved in cancer and immunological research. It will also be an important reference for students undertaking courses in Cancer Studies or Immunology.

contrasted with the "deme" within which there is free interbreeding, had seemed a contrivance, but the book convinced me of its biological interest. His concept of the "neutral pathway" is very stimulating. The examples are fascinating and show that the debate is about animals and not algebra, and the burying beetles in particular provide a challenge to ordinary, individual selection thinking.

A very valuable aspect of a book in which every main point is made with a model is that disagreements about it can be precisely stated. Wilson makes two controversial claims about his structured deme model which I believe to be mistaken. The first is that it "routinely" allows the evolution of a kind of altruism not predicted by the orthodox, individual selection model. The second is that species should act more in the interests of the community than the individual selection model predicts, because of "indirect effects". This second claim underlies the whole second half of the book. I believe that in making these important claims, Wilson has been misled by subtleties in his own algebraic formulations. The models supporting the two claims are very similar, and the following argument about "weak altruism" can be easily applied to "indirect effects".

To explain this rather important error, I will describe briefly the conclusions of the algebraic model. The effect of a trait is that its possessor has d more offspring while

every other member of the trait group has on average r more offspring. (Often either d or r will be negative.) Wilson first shows that in the individual selection model the trait will spread if $d > r$. This is because the trait group is the deme and a possessor of the trait must do better than average in the deme.

The condition for spread in the structured deme model depends on how the deme is divided into trait groups. If each group has exactly the same proportion of possessors of the trait — the identical groups case — then it is $d > r$. If possessors and nonpossessors of the trait are divided randomly into groups then it is $d > 0$. Wilson points out that there is a class of traits with $r > d > 0$ which will spread with random grouping but not with identical grouping, and these he calls "weakly altruistic". So far so good.

He goes on to identify the individual selection model with the identical grouping case of his structured deme model, and I believe this is an error. He concludes that the individual selection model is very special and implausible, and that we should expect to see animals behaving more generously towards each other than the individual selection model predicts. This is an important claim.

In both the individual selection model and the identical groups case the condition for spread is $d > r$, so how can Wilson be mistaken in identifying them? Because r must be interpreted differently in the two

cases. In the individual selection model every other member of the *deme* gains r but in the identical groups case only the other members of the *trait group* do. To see the significance of this difference, consider how the random groups case should be understood in terms of the individual selection model. The trait benefits each other member of the group by r , but it is the average effect on other members of the *deme* we need to use in the individual selection model. Wilson assumes that there are many groups in the deme, and so when the group members' r values are averaged over the whole deme the result will be very small. The condition for spread in the individual selection model then becomes nearly $d > 0$, even though r may be reasonably large. (In fact, the condition in the random groups case itself is only "nearly" $d > 0$.)

The random groups case, therefore, corresponds to the orthodox, individual selection model, and "weak altruism" is not, after all, a major contribution of structured deme theory to evolutionary thought. And, by a similar argument, neither is the concept of "indirect effects".

In conclusion, this book contains a very stimulating and clearly presented argument for using 'higher' levels of selection; but an argument which is seriously flawed. □

Alan Grafen is a member of the Animal Behaviour Research Group at the University of Oxford, UK.

Human internal clocks

J.T. Enright

The Circadian System of Man. By R.A. Wever. Pp.276. (Springer: Berlin, Heidelberg and New York, 1979.) DM98; \$53.90.

THIS monograph summarizes the results from some twelve years of experimental studies on human circadian rhythms, conducted in a unique underground facility at the laboratories of Professor Jürgen Aschoff in Bavaria. Data are available from more than 200 experiments, with an average duration of about a month each, during which the subjects — usually singly but sometimes in groups of 2 to 4 — lived in complete temporal isolation from the outside world. A few dozen comparable experiments have been conducted by others, elsewhere, but Wever's data base is such that this treatise can be taken as a definitive statement of present knowledge on the topic. Many of these results, which involve both free-running and entrained rhythms, have been previously published as a series of technical reports; nevertheless, there are conspicuous

advantages to having everything available in the present compact form: a well organized and systematic consideration of all the data, rather than interim, single-topic progress reports.

For me, the most interesting issue involved in these studies is the comparison of human circadian rhythms with those of other well studied vertebrates. Probably the most conspicuous difference is the extreme lability sometimes seen in the wake-sleep patterns of human subjects, superimposed on a more stable background of regular cycles in body temperature and other 'vegetative' rhythms. One hamster or mouse or finch seldom differs from the next by more than an hour in the free-running period of its wake-sleep rhythm, but some 20% of the human subjects had grossly non-circadian wake-sleep patterns (periods as short as 16 hours and as long as 60 hours). Subjects who thought they were living a normal routine occasionally undertook 'sleep deprivation' without even being aware of it: sustained wakefulness of up to 48 hours, while the temperature rhythm went on with its 24- to 25-hour oscillation. Another of the interesting differences between humans and other vertebrates, probably reflecting our domestication, is that lighting regimes — the overwhelmingly strongest synchron-

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izing agents for the circadian rhythms of other animals — apparently are only subsidiary in humans to the stronger effects of various sorts of social synchronization.

Wever himself has supervised all the massive programme of research described here, and the interpretations of the data are, of course, his own. Some of those interpretations are inevitably less convincing than others, and each reader will no doubt find certain issues about which he has reservations. It strikes me, for example, as over-interpretation, when the phase relationship between the body-temperature rhythm and the wake-sleep cycle is taken not just as a hint, but as proof that a subject's rhythm was accidentally synchronized by some unintended environmental cycle (p.38). I also wonder what to think about the implications of electromagnetic fields for the rhythms: small differences in mean period, correlated with shielding from natural electromagnetic fields, were statistically significant during the first 5 years of experimentation ($n=84$); but I find it distressing

to see that the effect was not reproduced during the subsequent 8 years of studies (p.96; $n=58$). An imposed 10-Hz square-wave electric field was associated with a shorter circadian period for 12 of 17 subjects tested (the other 5 excluded because of "internal desynchronization"); but in subsequent experiments, a 10-Hz sinusoidal field led to equivocal results (p.114). This difference can in principle be attributed to higher harmonics in the square-wave signal — or does it instead reflect on the reproducibility of the initial finding?

But these and similar questions are minor matters. Regardless of details in the interpretation, this monograph offers a wealth of data; it is an irreplaceable source book for those interested in the experimental study of human circadian clocks. □

J.T. Enright is Professor of Behavioral Physiology at the Scripps Institution of Oceanography, La Jolla, California.

Igneous petrology fifty years on

R. N. Thompson

The Evolution of the Igneous Rocks. Fiftieth Anniversary Perspectives. Edited by H.S. Yoder Jr. Pp.588. (Princeton University Press: Guildford, UK, 1979.) Hardback £19.30; paperback £8.40.

IN 1928 N. L. Bowen wrote his masterpiece *The Evolution of the Igneous Rocks*. This book caused a revolution in the philosophy and approach of igneous petrology by demonstrating that many aspects of the behaviour of magmas could be explained adequately by straightforward application of chemical principles, without the addition of the pinch or two of geological magic dust so frequently used by other theoreticians of Bowen's time. The 18 eminent petrologists who have contributed to the fiftieth anniversary volume are all the inheritors of Bowen's approach to magma chemistry. Each was asked to take a chapter of the original book and describe what has happened in that part of the subject during the following half century. As might be expected, there are great differences in the ways in which the various authors have approached their task. Some of them alternate extensive quotations from Bowen, or paraphrases of his ideas, with balanced summaries of the present state of knowledge on each theme. In contrast, a few of the contributors have been unable to resist the temptation to use their chapters almost solely as vehicles for summarizing their own work and reiterating their own views; in one article

there is no mention at all of either Bowen or his book.

Taken as a whole, the many excellent articles far outweigh the few others. The chapters that I found particularly interesting were on silicate liquid immiscibility (Roedder), crystal accumulation and sorting (Irving), siliceous potassic glassy rocks (Stewart), volatile constituents (Burnham) and petrogenesis (Wyllie). There are also many sections of valuable data and discussions in other chapters and I anticipate that this book is one which almost every working igneous petrologist or geochemist will wish to buy. On the other hand, it is probably of only limited use to the wider readership of students. The reason for this lies in the terms of reference of the volume. Rigorous adherence by the contributors to the themes in igneous petrology which Bowen chose in 1928 has inevitably led to omission, or virtual omission, from the 1979 book of any subjects which were neglected or completely unrecognized 50 years ago. This policy leads to a curious overall impression in the book, because many aspects of current igneous petrology are discussed in detail, whilst other important themes, such as crystallization kinetics, and trace-element and isotope geochemistry, are almost completely omitted. The irony is that, if Bowen had rewritten his own book in 1979, these are exactly the sorts of forward-looking applications of chemical principles to igneous petrology that he would have emphasized. □

R. N. Thompson is Lecturer in Geology at the Imperial College of Science and Technology, University of London, UK.

Selective electrodes

J.A.W. Dalziel

Working with Ion-Selective Electrodes. By K. Cammann. Pp.225. (Springer: Berlin, Heidelberg and New York, 1979.) DM 76, \$41.80.

THIS monograph, a worthy successor to others that have appeared, is a translation from a second German edition. It reviews 447 publications up to 1977. The author, after experience in the applications department of an instrument manufacturer, claims an understanding of the problems that face the users of ion-selective electrodes. He considers that their difficulties arise mainly from lack of knowledge and remedies this in two ways — first, with a theoretical treatment to serve as reference in understanding the action of electrodes in potentiometry generally, and then by offering practical advice on their construction, mode of operation and a survey of their present range of applications.

Particular emphasis is given to consideration of pitfalls in the reproducible measurement of electrode potentials, with advice on reference electrodes, buffers and liquid junction potentials directed towards stable e.m.f. measurements of high impedance cells. The different categories of ion-selective electrodes (solid-state glass, homogeneous and heterogeneous membranes, supported ion exchanger and neutral carrier membranes, and electroactive coatings, gas-diffusion and bio sensors, but without the glass pH electrode which is intentionally omitted) are all described in turn with full practical details, including methods of construction.

The second half of the book has sections on techniques and applications. Besides calibration and the direct measurement of activity (concentration) under static conditions, much space is devoted to titration procedures with indicator electrodes, using the Gran extrapolation and other methods which allow for the slow response of some electrodes near the end point. Whether such methods would be chosen for routine situations is questionable. The short section on continuous measurements in industry and environmental research should be more interesting, but in fact the progress reported is rather disappointing when one remembers that it is over 15 years since 'new' ion-selective electrodes were made. For the future, the important step will be to discover better ways of interfacing the electrolytic species in sample solutions so that they react directly and selectively with electronic charge carriers within semiconducting electrodes. □

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INSERM CONFERENCES INSERM CONFERENCES INSERM CONFERENCES INSERM CONFERENCES INSERM CONFERENCES

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The INSERM CONFERENCES 1980 will be held at the Domaine de Seillac, near Blois (180 km from Paris), during the month of September (full address : Domaine de Seillac, 41150 Seillac, France). Each conference will last three and a half days, from Sunday evening (departure from Paris) to Thursday afternoon. Working sessions will be held from 9 a.m. to 12.30 p.m. and from 5.30 p.m. to 8 p.m. On free afternoons, participants will have a wide choice of leisure activities at the Domaine de Seillac and in the surrounding area (Tennis, Table-tennis, bicycling, visit to the Castles of the Loire).

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- GLADWELL, I. and WAIT, R. (ed. by). A Survey of Numerical Methods for Partial Differential Equations. Pp.x + 424. ISBN-0-19-853351-9. (Oxford: Clarendon Press, Oxford University Press, 1979.) £13.50.
- GRAHAM, Alexander. Matrix Theory and Applications for Engineers and Mathematicians. Pp.295. (Chichester: Ellis Horwood Limited, 1979.) £15.75.
- KNOPFMACHER, John. Analytic arithmetic of algebraic function fields. Pp.v + 130. ISBN-0-8247-6907-4. (New York and Basel: Marcel Dekker, Inc., 1979.)
- ROBERTS, F. S. Measurement Theory with Applications to Decision-making, Utility, and the Social Sciences. Encyclopedial of Mathematics (Massachusetts, London, Amsterdam, Ontario, Sydney, Tokyo: Addison-Wesley, 1979.) \$24.50.

Astronomy

- BURTON, W. B. The Large Scale Characteristics of the Galaxy. Proceedings of IAU Symposium No. 84. Pp.xviii + 611. ISBN-90-277-1029-5 hardback ISBN-90-277-1030-9 paperback. (Dordrecht, Boston, London: D. Reidel, 1979.) Dfl. 140 US\$73.50 hardback; Dfl. 70 US\$36.85 paperback.
- LANG, Kenneth R. and GINGERICH, Owen (ed.). A Source Book in Astronomy and Astrophysics, 1900-1975. Pp.xx + 922. ISBN-0-674-82200-5. (Cambridge, Massachusetts and London: Harvard University Press, 1979.) \$50.00.
- KRUGER, A. Introduction to solar Radio Astronomy and Radio Physics. Pp.xv + 324. ISBN-90-277-0957-2 hardback; ISBN-90-277-0997-1 paperback (Dordrecht, Boston, London: D. Reidel, 1979). Dfl. 95 US\$49.95 hardback Dfl.45 US\$23.60 paperback.

Physics

- CHARNEY, Elliot. The Molecular Basis of Optical Activity. Pp.x + 364. ISBN-0-471-14900-4. (New York, Chichester, Brisbane, Toronto: John Wiley & Sons, 1979.) £16.80.
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- PALMADESSO, P. J. and PAPADOPOULOS, K. (ed.) Wave instabilities in Space Plasmas. Proceedings of a Symposium organized within (Dordrecht, Boston, London: D. Reidel, 1979.) Dfl. 75 US\$39.50.
- PEIERLS, R. Surprises in Theoretical Physics. Pp.viii + 166. ISBN-0-691-08241-3 hardback ISBN-0-691-08242-1 paperback (Princeton, New Jersey: Princeton University Press, 1979.) £8.60 hardback £2.20 paperback.
- ZIMAN, J. M. Principles of the Theory of Solids. Pp.xviii + 435. ISBN-0-521-08382 hard covers ISBN-0-521-29733-8 paperback. (Cambridge and London: Cambridge University Press, 1979.) £6.50 paperback.

Chemistry

- ABRAHAM, R. J. (Senior Reporter) Nuclear Magnetic Resonance vol 8. A Review of the Literature published June 1977 and May 1978. Pp.xxiv + 351. ISBN-0-85186-322-1. (London: The Chemical Society, 1979.) £28.00 \$72.00.
- AYLETT, B. J. Organometallic Compounds vol. 1 part 2 groups iv & v. Pp.xi + 521. ISBN-0-412-13020-3. (London: Chapman & Hall, 1979.) £32.50.
- BOSCHKE, F. L. (ed.). Topics in Current Chemistry; volume 84 Bioactive Organo-Silicon Compounds. Pp.iv + 146. ISBN-3-540-09347-8. (Berlin, Heidelberg, New York: Springer, 1979) DM 88 \$48.80.
- BOTTLE, R. T. Use of Chemical Literature. Pp.xiv + 306. ISBN-0-408-38452-2. (London and Boston: The Butterworth Group, 1979) £15.00.
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10 April 1980

Eleventh hour for biotechnology in Britain

WHETHER or not the National Enterprise Board decides to, or is allowed to, set up a biotechnology company, the Spinks report published last week was right to have suggested that the Board consider the possibility (see page 502). There is little doubt that Britain has long been in need of companies prepared to risk investment in biotechnology. The only question now is whether it is too late.

As far back as 1975 it was obvious to a certain number of UK scientists that there was considerable commercial potential in new techniques emerging from molecular and cellular biology. In particular it was recognized that the technique of recombinant DNA might be used to harness bacterial and other cells to the production of pharmaceutically active polypeptides. Gene manipulation was also seen to have much to offer in the improvement of strains of microorganisms employed in fermentation and waste disposal. Bacterially cloned genes could also be foreseen as valuable in the diagnosis and therapy of congenital diseases. Equally there was early, if limited, recognition of the commercial potential of hybridoma cells which produce single antibodies — which are of considerable value in radioimmunoassays and other medical diagnostic techniques.

It was with some chagrin that scientists in Britain, including those responsible for some of the key discoveries, watched entrepreneurs in the US start up a series of small venture capital businesses aimed to cash in on the new techniques, whereas nothing equivalent emerged in the UK. It still has not.

Nor, with a few exceptions, have established UK industries been quick off the mark to exploit the new techniques. Most companies have seemed content to bide their time rather than to fund research that may have looked promising but whose value would not be proven without long term investment. In the event, progress abroad has been much faster than was anticipated. Although real fortunes, as opposed to paper ones, are not yet being made from the new biotechnology, there can be no doubt that they will be. And yet there is still a distinct lack of interest from UK industry.

With neither established nor new companies to fund and benefit from UK scientific expertise, universities and organisations such as the Medical Research Council and the Agricultural Research Council find it difficult to exploit commercially the discoveries of their scientists. Could their requirements be met by a publically financed company?

Any such proposal will meet three hurdles. The first is time; is it too late to set up such a company? Here the answer must be a qualified no. It is probably too late to think in terms of producing hormones and vaccines by means of genetically-manipulated bacteria; those are first generation applications already far advanced. But there is no shortage of second generation applica-

tions, particularly those involving fungal, mammalian and plant cells, with which a newly formed company might hope to leapfrog the early starters.

The second hurdle is manpower. While British industry and entrepreneurs have been dallying, many of the academic scientists have become involved with US-based, or US-financed, venture capital companies. Other British scientists have resisted all approaches and will probably continue to do so. It is therefore dangerously late to be recruiting scientists to the cause. Nevertheless there does appear to remain sufficient untapped expertise, particularly in the laboratories of those research councils most keen to see a company founded, to make the project worth pursuing.

The third hurdle is the Conservative government which, particularly with its present leaders, strongly favours private over public enterprise. But faced with a demand unfulfilled by the private sector no government should stand endlessly on principle.

If all three hurdles can be jumped, there remains the question of how best to set up a public company. The obvious way, as recommended in the Spinks report, is through the National Enterprise Board (NEB) and/or the National Research Development Corporation (NRDC). Although the NRDC already has some expertise and experience in the patenting and licencing of biotechnological processes, there are drawbacks to its further involvement. In the first place whereas it commonly gives a half share of royalties to any university from which a patented discovery has emerged, it is constitutionally unable to pass royalties back to research council laboratories. Secondly it is willing only to fund research with a clear commercial potential. Thirdly, and despite its having taken out such important patents as those on cephalosporins and interferon (unfortunately time-expired except in the US, where it runs until 1989), the NRDC has not consistently spotted the winners. One glaring mistake is its 1975 decision that hybridomas had no obvious commercial applications.

Given the drawbacks of the NRDC, there is a good case for the NEB to found a biotechnology company. The chief attraction of this proposal is simply that a new company, devoted to a relatively narrow venture, would be able to avoid most of the problems associated with the NRDC. Clearly the success of such a company would hinge crucially on the calibre of its staff, their ability to pick areas in which they could mount an effective challenge to those companies already in the business, and the degree to which productive links could be built and maintained with academic scientists.

None of this will be easy. It will become even less easy as time passes. Therefore if, as we believe, the NEB should start a biotechnology company in the UK it should do so now. □

United States

Budget cuts stop research growth

Delays in space science missions, construction of a heavy ion accelerator, and a scheme to improve university research facilities are among the victims of last week's US budget cuts. **David Dickson** reports

It could have been worse — and for a time it looked as if it was going to be. But when President Carter announced last week where the axe was to fall in his attempt to balance the federal budget, it became clear that the growth enjoyed by basic research for the past few years has, at least temporarily come to a halt. Indeed the first indications are that the cuts, reducing expansion to below the expected inflation level, will mean a net contraction in overall funding.

Hardest hit will be the National Science Foundation, which had expected a 17.7% growth in its budget in 1981, but has had this reduced by \$74 million to an 11% increase. Close behind is the National Aeronautics and Space Administration, although the space shuttle programme has been protected from cuts, largely because of its commercial and military significance.

In other agencies the impacts have been less severe. The Department of Energy, for example, has had the growth in its basic research budget cut from 13.4% to 12.2%. And at the National Institutes of Health, where possible cuts of up to \$500 million had initially been discussed (largely because it is one of the few areas of discretionary spending in the health budget), it was finally agreed to reduce the 1981 basic research budget by only \$35 million out of \$1,704 million.

Dr Frank Press, Director of the Office of Science and Technology Policy, told *Nature* last week that in deciding where the President's original budget proposals for 1981 should be reduced, it was decided to aim primarily for demonstration and development projects rather than basic research support.

"New initiatives were slowed down but not dropped if they were philosophically important to us" said Dr Press.

Some of the more detailed cuts are: ●NASA: total research and development budget reduced from \$5.7 to \$5.5 billion. The space science budget, previously planned to rise from \$600.9 million to \$668 million, will now fall to \$561 million.

In addition to a two-year delay in the Solar Polar Mission, future experiments in physics, astronomy, life sciences and environmental observation planned for flight on Spacelab missions will be delayed for one to two years.

These cuts will leave unaffected two new starts proposed for the 1981 budget, namely the Gamma Ray Observatory, and the National Oceanic Satellite System. Nor

will the cuts effect either the Galileo Mission or the space telescope, both initially under threat when possible cuts of up to \$760 million were being discussed.

However funding for the Earth Radiation Experiment, NASA's proposed contribution to the National Climate Plan, will be delayed for one year. And the agency will also be cutting back on cosmic ray research, as well as terminating relativity research and the analysis of data from earlier pioneer missions (although the latter will be kept for future analysis).

●Department of Energy: the high energy physics programme will be cut by \$4

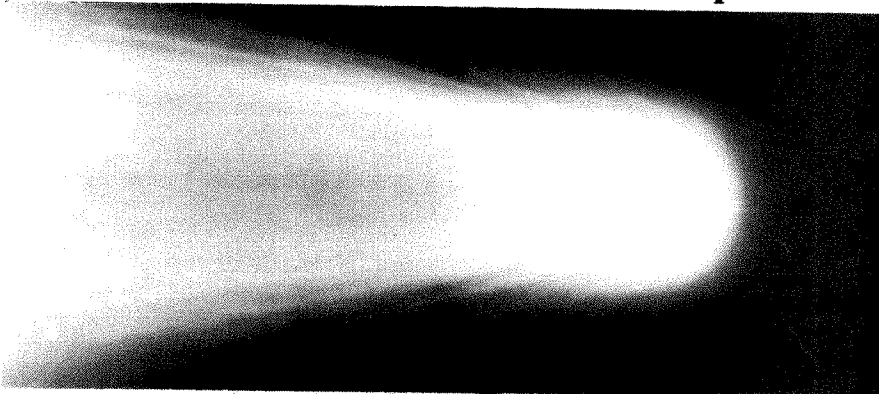
million from its original proposed level of \$359 million. This cut will be absorbed at Brookhaven, where the construction of Isabelle is already experiencing delay due to problems with the superconducting magnets.

A similar sum is being cut from the nuclear physics budget by deferring for one year construction of the Heavy Ion Beam Argonne tandem accelerator system.

In addition, \$3million inserted into the budget to support the setting up of advanced energy research centres at leading US universities (an idea proposed only last autumn by Dr David Saxon, of the University of California) has been dropped.

●National Science Foundation: within the NSF two strategies have been pursued. Firstly to preserve as much as possible of core responsibilities for basic research.

Delay for space mission with Europe



Halley's comet: delays to the solar polar mission mean hope for a cometary mission for Europe

AMONG the organizations to be affected by the US budget cuts is the European Space Agency, which is collaborating with NASA on a project to send two spacecraft in complementary polar orbits around the Sun, known as the International Solar Polar Mission.

The Carter administration, which included the mission as a new start in last year's budget submission to Congress, is now proposing to save \$43 million in 1981 (compared to \$83 million proposed in January) by delaying the launch of the mission from 1983 to 1985.

The delay will cause serious programming problems for ESA, whose space science projects are already on a tight schedule, and which last summer signed a \$56 million contract with Dornier to construct the European spacecraft by 1983.

The agency now has to decide whether to allow construction to be completed as planned, and subsequently put the spacecraft in mothballs; or to slow down construction work, hoping that the money

saved can be absorbed by other projects and will not have to be returned at the end of the year.

Either strategy will make the project more expensive than when it was approved.

This particular cloud may, however, prove to have a silver lining. For some of the money freed from the ISPM might be available to support a fly-by mission to the comet Halley, which would have to be launched at the end of 1984.

After NASA had informed ESA in December that it was unlikely to be able to support a joint mission to Halley and the comet Tempel 2 because funding for a solar electric propulsion system had been deleted by the Office of Management and Budget, ESA's scientific programme committee agreed last month that it would be prepared to consider a European solo mission, providing the costs are under 80 million accounting units (about \$110 million).

Detailed proposals for such a mission will be considered when the committee meets again in August. □

And secondly to maintain the direction of new initiatives introduced into the 1981 budget as a result of last year's domestic policy review of industrial innovation, even though at a reduced level.

In areas of project support, each field will be scaled downwards, although the emphasis will be maintained on shifting the balance of support back to physics and engineering from the life sciences. Thus the total for mathematical and physical sciences will be reduced by \$4.4 million (leaving a 14.5% increase over 1980), and for biological and behavioural sciences by (\$4.1 million, leaving a 6.8% increase).

The increase in funding for university-industry cooperative research projects will be reduced by \$5.1 million, from \$13 to \$7.9 million above this year's level of \$7 million. And support for small business innovation and industrial technology, initially scheduled to be raised from \$7.3 million to \$18.2 million, will now only increase to \$9.2 million.

Another new initiative to be cut back is the Ocean Margins Drilling Project. This is to be jointly funded with the oil industry, and had been scheduled to receive \$10 million in 1981, but the figure will be cut back to \$5 million.

More money has been saved by putting off detailed design studies of a 25-metre diameter millimeter wave telescope, which would have been scheduled for funding in 1982, as well as plans to purchase a new coastal research ship.

The NSF has also decided to defer a proposal to award \$14.3 million on a matching grant basis for improving university research facilities. There will also be a \$10 million cut in science education programmes.

Finally, the Foundation is cutting back on its joint scientific programmes with both the USSR and the China (the latter introduced for the first time in 1931). In particular, a programme with Soviet scientists on chemical catalysis, and a working group in scientific and technical information, will both be terminated, and these, together with cutbacks in exchanges through the National Academy of Science, will reduce the US/USSR budget from \$3.2 to \$1 million. Scientific cooperation with China will have its support reduced from a proposed \$2 million to \$1.5 million.

● **Defense Department:** the Department of Defense is the one agency to have escaped significant budget cuts. Precise figures have yet to be agreed, but it is expected that the department's original proposal will be cut back by between \$10 and \$20 million. This will still permit an increase in military support for basic research of about 15% — well above the expected rise in costs.

The big question now is how Congress will react. In general the cuts from its original budget proposals recommended by the administration are in line with those supported by Congressional budget committees, but there could be more surprises in store. □

Energy choices: the cultural assumptions

THE debate surrounding the report of the National Academy of Sciences Committee on Nuclear and Alternative Energy Systems (CONAES) continues to reveal as much about different perspectives on the energy problem as it throws light on possible solutions.

Latest contribution to this debate is the report of a panel set up by the committee to study the "lifestyle" implications of different projected energy scenarios. Published by the Academy last week, the report carries the emphatic message that "energy is a social, not a technological issue".

A society's demand for energy must be seen as part of its general organisation of preferences. Yet in many discussions of the factual basis of energy problems, the cultural and social contexts tend to be left implicit; and research on social and cultural factors influencing energy decisions has consequently received "remarkably little attention".

"We know something about the technology of energy, but much less about the agents: the experts, the interest groups, the public," says the report, which recommends that more research is needed on the social organisation of energy experts.

"Do nuclear physicists and engineers, for example, dominate the latecoming biologists, health physicists and social scientists?" it asks. "What is the relationship between the experts who estimate the probabilities of harmful events and those who assess their consequences?"

The panel which produced the report was chaired by Dr Laura Nader Professor of Anthropology at the University of California, Berkeley. In publishing it, the Academy points out that it is intended as a "supporting paper" to the full CONAES report, and that it has not gone through the "normal critical review" for such reports — although adding that it has been "subjected to a thorough and expert peer review for accuracy, consistency and clarity."

The bulk of the report concentrates on some of the possible implications of two future energy scenarios that might be expected in the year 2010. The first assumes a level of energy consumption in the US of 71 quads, equal to estimates of consumption in 1975 when the study was started.

This level of energy demand, says the report, could be achieved without any significant changes in attitudes, and would still involve increases in amenities, with improvements "roughly consistent with those that have occurred in recent decades."

The second scenario is based on the assumption that society agrees to cut its consumption to 53 quads — and is offered by the panel not as a prediction of what is likely to happen, but as a way of focusing on the relationship between energy demand and cultural values.

This scenario, says the panel, implies a significant shift in attitudes, including a decentralisation of work, and high value being placed on thrift and self-reliance. In what it characterises as a "high technology, low energy consumption" society, the aim would not be to turn back the clock, but to develop new technologies — such as advanced automobiles, or microprocessor building and process control techniques — aimed at improving the quality of life under the new energy constraint.

Pointing out that societies such as Sweden's already exist on much lower energy consumption than the US, the panel suggests that its scenario would be feasible, and would reflect the needs of a participatory democracy which might be challenged by increasingly centralised technological systems (such as those involved in the use of nuclear power).

"The trends toward tightly meshed technological systems characteristic of the 1970s are reversed in the 53 quad society, increasing the likelihood that most of the system can survive if a part of it is severely damaged," the report says.

In its conclusion, which stresses the need to integrate long-term energy planning with other kinds of social planning, the panel emphasises that there is nothing improper in the fact that political and social decisions about resource use extend beyond the market place and are influenced by subjective values and judgments.

"What is alarming is that experts often pretend to be able to perform objective analyses on energy systems or energy users because they are unwilling to acknowledge the values on which their decisions or analyses were made," it says.

Perhaps unsurprisingly, few of the more outspoken conclusions were included in the final report of the full CONAES committee. And this itself has provoked a reaction from Dr Nader, who says that her panel was told that its work "was not quantitative enough", and should include "more tables and less prose."

"The criticisms raised old disagreements about the use of qualitative and quantitative methods and illustrate how difficult it is in general for many scientists to incorporate findings from disciplines which could contribute to more intelligent discussions of unbounded problems" says Dr Nader. □

UK biotechnology

R&D attitudes wrong, says report

The "Spinks report" on the future of biotechnology in the UK was finally published last week. Robert Walgate reports

"THERE is a clear pre-development gap in R&D funding in Britain" Dr Alfred Spinks, chairman of a working party whose report* on UK biotechnology had just been published told a press conference last week. There is too little adventurousness, "a lack of gambling money". This is what is needed now for biotechnology in the UK, though it was clear that "one cannot transform the UK into an entrepreneurial state like California".

Dr Spink's report, prepared by the seven-man working party, says that "the present structure of public and private support for R&D is not well-suited to the development of a subject like biotechnology which, at the moment, straddles the divisions of responsibility both among government departments and among research councils and the arbitrarily defined fields of applied and fundamental research".

The recommendations and criticisms in the report are substantially the same as those included in a draft version detailed in *Nature* on 24 January (page 324). A joint committee for biotechnology should be set up to coordinate the biotechnical work of the five research councils, and a parallel interdepartmental steering committee set up for government departments.

Research councils should double their present commitment to biotechnology to a level of at least £3 million a year, and government departments should commit some £2.5 million. The National Enterprise Board and National Research Development Corporation should consider setting up a "research-oriented biotechnology company" with £2 million a year for five years.

John Ashworth, Chief Scientist at the Cabinet Office, who coordinated the working party, said "after five years we would expect industry to come in and invest in the company. If they don't, the venture will have failed."

"People are the main problem, not money" said Ashworth. The estimated spending on a biotechnical firm had included £1 million a year overheads, plus 50 scientists at £10,000 salary plus £10,000 research expenses. The 50 people could be found without difficulty, despite the increasing recruitment to foreign firms, thought Dr Spinks. "And we should also look abroad for talent".

The report supports the expansion of centres of excellence in the subject in universities. "A minimum of 20 new teaching and research posts should be created over the next five years with a capital investment of around £2 million" for lab facilities, says the report, and careful attention should be paid to the

training of an appropriate workforce.

● Professor Brian Hartley, a member of the Spinks working party, said last week that it was too late to consider setting up a firm to exploit British biotechnical talent, as recommended in the report.

Professor Hartley is a British member of Biogen, a US and Canadian-funded genetic engineering contract research body on which the UK firm, code-named 'Greenfields' by the National Enterprise Board, might be modelled. Biogen announced the cloning of interferon in the bacterium *E. coli* a few weeks ago, and completed a laboratory in Geneva last December; and recently it has been recruiting new staff.

The problem a new UK-based firm would face would be to find enough good staff," said Hartley. "A month ago they were available. Now they've joined us."

Hartley would not give precise figures. But "a few tens" have been recruited for the Geneva laboratory, to be compared with the 50 required for the UK firm. □



*Biotechnology, HMSO, £3

Crucial NEB decision due today

IF all goes according to plan, the UK National Enterprise Board (NEB) will today be taking a crucial decision on whether or not to seek ministerial approval to sink at least £10 million of public funds into British biotechnology.

The idea that the NEB should become involved in biotechnology receives impetus from last week's publication of the Spinks report which recommends that public funds be used to set up a research-orientated biotechnology company in Britain. However, the NEB were already investigating the possibility last summer. And last December, Mr Benjamin Lewin, editor of the journal *Cell*, was in the UK sounding out the opinion of British scientists on behalf of the NEB.

Although the details of the proposal are unclear, it is believed to favour both the establishment of a small specialist company and the funding of promising research in academic laboratories. In that way it might be possible to make the most of the proposed investment of £10 million, over a five-year period.

If the NEB decides to proceed with the British proposal, it is almost certain to have to seek approval from the Secretary of State, Sir Keith Joseph. Strictly speaking, the NEB would not need to do this because it was delegated the authority to invest up to £10 million by the Labour government which set it up in 1975. The Conservative government is less well disposed towards the concept of the NEB, (particularly

because it was often used to rescue the lame ducks of British industry) and is in the process of limiting its role and reducing to £5 million the sum it can invest without ministerial approval.

Likely recipients of any funds made available by an NEB-owned company would include the Agricultural Research Council's Plant Breeding Research Institute, the Imperial Cancer Research Fund laboratory in London and such Medical Research Council (MRC) laboratories as the Cell Immunology Unit in Oxford and the Laboratory of Molecular Biology in Cambridge. Within each of these laboratories there is considerable expertise on two of the techniques which underpin the current wave of biotechnology, namely genetic engineering through recombinant DNA techniques and monoclonal antibody production from hybrid cells.

Under pressure from the Treasury to take more seriously than it sometimes has done the possibility of commercially exploiting scientific discoveries made in its laboratories, the MRC has recently reminded its senior scientific staff of their obligation to draw to the Council's attention any results of commercial potential. In particular the MRC is interested in the distribution and marketing of monoclonal antibodies derived from hybrid cells by the technique devised in 1975 by Dr Caesar Milstein and Dr George Kohler in the MRC Laboratory

of Molecular Biology.

The potential market value of monoclonal antibodies and the lack of a satisfactory route for commercially exploiting them is well known to the MRC. At the end of February it asked its scientific staff to provide full information on the availability and characterisation of hybrid cell lines as they were produced. At the same time the MRC said that it was exploring the means by which such cells and the antibodies produced from them could be distributed and marketed in a coordinated way.

Questioned whether this represented a new departure in MRC policy, Dr James Gowans, MRC secretary, said that the policy was not new but that, under pressure from the Treasury, the possibilities of commercial exploitation were being looked at more aggressively. Many monoclonal antibodies produced in MRC laboratories are already being marketed through the small UK firm of Sera-Lab Ltd, with a proportion of the sales revenue being channelled back to the laboratories. But, said Gowans, the antibodies marketed by Sera-Lab tended to be those of marginal commercial value. For the development and marketing of antibodies of high commercial value either because of the anticipated volume of sales or because of the rarity of the cell hybrids from which the antibody was derived, Gowans would welcome the existence of a company of the kind that the NEB might start.

Peter Newmark

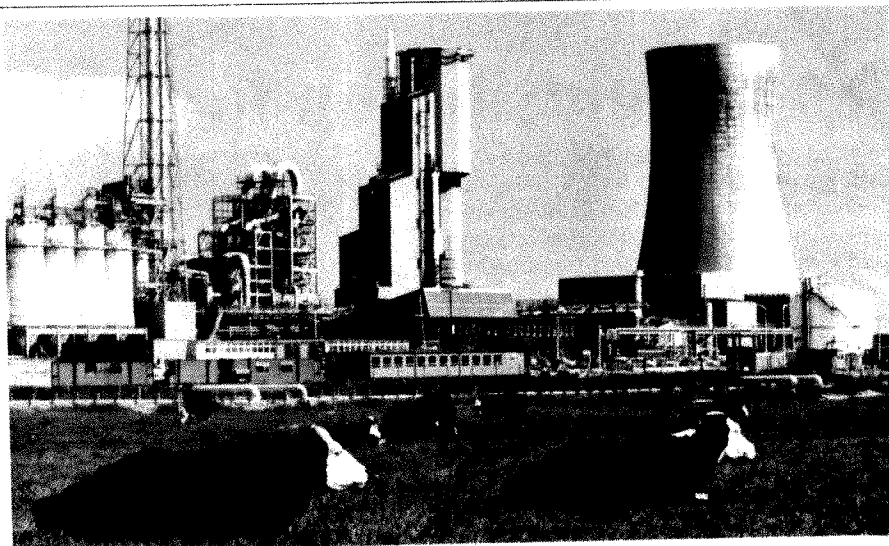
Spinks offers "too little"

THE chairman of Cetus Corporation, America's first and largest contract biotechnical enterprise, believes that £2 million a year for five years is far too little to invest in a new biotechnical firm — as envisaged in the Spinks report. Pressed as to what would be a reasonable investment, he suggested \$50 million over five years would be the minimum "if you are not to waste your cash".

The Cetus chairman, Dr Ronald Cape, was speaking at a conference on biotechnology in Rotterdam at the end of last month. He eschews government funding or work undertaken in universities outside Cetus's direct control. "We do our work in our labs" he said.

Returns will be long in coming, thinks Cape: no genuine profit for 5 to 10 years. Nevertheless, Cetus "is now investing millions of dollars of its own money, and comparable amounts on behalf of major company clients," in biotechnology.

New companies like Cetus will move biotechnology forward, thinks Cape, but new companies are not necessarily small. Cetus has 250 employees — "the largest collection of molecular biologists in the world" — and 18 projects in five industries, all with potential markets of billions of dollars. "Biotechnology is no longer cheap" says Cape. □



Two types of fermenter: cows outside ICI's single cell protein plant

Single cell protein organism improved

MICRO-ORGANISMS selected by the British firm Imperial Chemical Industries (ICI) to produce single cell protein (SCP) have been made more carbon-efficient by an application of recombinant DNA techniques, the firm announced last week.

ICI recently established the world's largest SCP fermenter — more than 50m high — using a methanol-consuming organism known as AS1. But the process has been under constant market pressure from agriculturally-produced soya-bean protein (both are used as animal feed supplements) and so ICI has been at pains to reduce the basic cost of its SCP. One obvious target was to improve the efficiency with which AS1 converts the costly methanol feedstock into protein.

Protein requires nitrogen, and AS1 gets its nitrogen from ammonia. But ICI discovered that AS1 assimilates the ammonia by an energy-inefficient pathway — one using the enzyme glutamate synthase to convert ammonia to glutamate. The energy for this comes ultimately from the oxidation of methanol to carbon dioxide, so the result is an excessive consumption of methanol.

But some organisms have a different pathway, using glutamate dehydrogenase, which is less able to scavenge small amounts of ammonia but is more energy-efficient (and thus carbon-efficient). The possibility thus arose that the glutamate synthase gene in AS1 could be deleted, and the glutamate dehydrogenase gene substituted for it.

Luckily the well-studied *Escherichia coli* has the right gene, and Dr John Windass of ICI Corporate Laboratory was able to excise its gene and insert it into AS1. The greatest problem turned out to be how to isolate an AS1 mutant which had a deletion in the glutamate synthase gene: such mutants die immediately. However, ICI discovered a temperature dependent mutation — one where the glutamate synthase fails to operate above 37°C — and

Windass inserted the dehydrogenase gene into this mutant.

Above 37°C this modified strain uses the dehydrogenase pathway for assimilating ammonia; and over a two-months period the strain has proved to be stable.

Dr Windass, speaking at a recent biotechnology meeting in Rotterdam, claimed that the engineered strain produced a "significant" improvement in carbon efficiency. Questioned whether a 3 to 5% improvement would justify the long toxicological studies that would be required if the strain was eventually to be employed, another ICI representative, Dr Peter Senior remarked that it would.

Toxicology studies are underway on the new strain, but it would be "at least five years" before it could be used. The strain could be adopted in the newly commissioned SCP plant, but there its efficiency would be sub-optimal, said Senior. "You have to design your plant around your bug".

In fact, Senior believes that the future of biotechnology will not be all plain sailing. "All the easy things have been done", he went on. "Biotechnology is grossly overselling its potential, and there is little likelihood on both scientific and economic grounds that we are staring a revolution in the face".

Conceptually the simplest biotechnical process was the growing and harvesting of single cell organisms, said Senior, yet "from my experience the single cell protein processes that have been developed at ICI have stretched the imagination and innovative skills of all those involved in their development to a degree that the conventional chemical industry has not experienced before".

The ICI single cell protein process was first envisaged in 1968; in 1976 a sum of £40 million was sanctioned for the construction of a large plant, which is now on the point of production.

Robert Walgate

United Kingdom

No conclusion on low level lead hazards

A government report, published last week, has provoked strong criticism from the lobby demanding reductions in the amount of lead added to petrol. The report, *Lead and Health**, concludes that lead in air is not the most significant contributor to lead contamination in people: food and water are more important. "In the vast majority of the population", it says, "airborne lead, including that derived from petrol, is usually a minor contributor to the body burden".

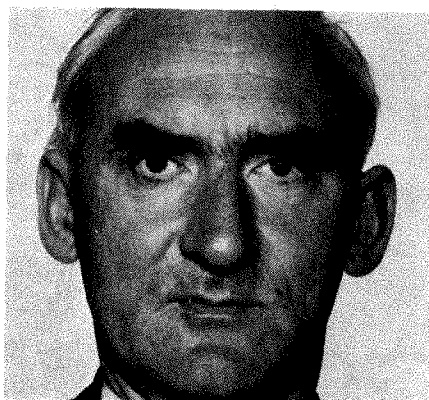
The working party on lead, set up in November 1979 under the Chairmanship of Professor P.J. Lawther, head of the MRC Toxicology Unit at St Bartholomew's Hospital, to prepare the report, concludes that it has only been able to satisfy one of the objectives required by its terms of reference — that of assessing the role of lead from petrol in relation to the other sources of lead in the environment. Its other aims — "to review the overall effects on health of environmental lead from all sources and, in particular, its effects on the health and development of children" — have been more difficult to achieve.

The toxicity of heavy exposure to lead is well known. The main controversy is over whether or not exposure to fairly low levels can effect health, especially the mental health of children. Several studies, in the US and Europe, suggest that children exposed to levels of lead which would not be high enough to result in the conventional signs of lead poisoning, have lower IQs and more behavioural problems than children who have not been exposed, or only very slightly.

The working party tried to meet the second part of its terms of reference by reviewing these studies. It concludes that there is no evidence of "deleterious effects at blood lead levels below about 35 $\mu\text{g}/\text{dl}$; neither is there any doubt about the serious consequence of blood lead levels above about 80 $\mu\text{g}/\text{dl}$; at intermediate concentrations, however, it says that the evidence is still very uncertain.

Many of the studies on the intelligence and behaviour of children are criticised in the report for not giving full details of their methodology. It says that in many of them, conflicting results have meant that the working party has not been able to come to any "clear conclusions concerning the effects of small amounts of lead on the intelligence, behaviour and performance of children". It recommends that further and more careful studies should be done: in particular better tests for examining intellectual function and behaviour in small groups of children are needed as is more research on the effects of pre-natal exposure on subsequent development.

Nevertheless, the report recommends



Professor P.J. Lawther, working party chairman

that children with blood lead levels above 35 $\mu\text{g}/\text{dl}$ should be followed up to identify the source of lead exposure.

Most cases of heavy contamination, the report concludes, are unlikely to result from exposure to unusually high concentrations of lead in air, although there will be local "hot spots" where airborne lead concentration are high and make a major contribution to overall body burden. Such areas should be identified and steps taken to minimise the hazard. The most likely sources of heavy exposure, however, are tap water, lead in paint and lead in imported cosmetics. The report recommends that a programme to detect lead in paint coatings accessible to young children be set up and that local authorities and water boards be encouraged to reduce lead in tap water in badly affected areas. The import and use of lead containing cosmetics should be discouraged, it says, and potential users should be made aware

of the hazard.

Other recommendations made by the report are the elimination of lead from manufactured foods — this is particularly a problem with canned foods — the development of guidelines for acceptable concentrations of lead in soil — more research also needs to be done on how plants take up lead from soil — and better education of the public on the hazards of lead especially to small children.

On the controversial topic of emissions of lead to the air from traffic, the report recommends that it should be progressively reduced and that the annual mean concentration of lead in air should be kept at less than 2 $\mu\text{g}/\text{m}^3$ in places where people spend a lot of time. Measures to achieve these limits may include "reduction of emissions, the relocation of industry or housing or traffic management schemes".

The report has been criticized by the anti-lead lobby. Parents Against Lead issued a statement at the time of the report's release saying that it had not taken into account evidence linking birth defects with lead contamination of the mother neither had it considered research already done showing that much of the lead in food comes from the air. To suggestions that the working party had been swayed by the oil industry, Professor Lawther said that each member of the party had their own scientific reputation to maintain and they had struggled to produce a fair scientific assessment of the subject.

Judy Redfearn

Lead and Health: the report of a DHSS Working Party on lead in the Environment HMSO £4.50

Dual support system for review

THE 'dual support' system whereby UK universities pay for buildings, services, technicians, basic laboratory equipment and salaries while research councils pay short-term grants for specific research is to be questioned by a new 7-man working party which will report ultimately to the Department of Education and Science.

The working party is "to review the current arrangements for the support of university research in the natural and social sciences". It is drawn from the Advisory Board for the Research Councils, which advises government on the allocation of the science budget among the research councils, and the University Grants Committee which provides universities with their basic running funds. The party will be chaired by Sir Alec Merrison, Chairman of the ABRC; other members include Dr Edward Parkes, Chairman of the UGC, and Sir Geoffrey Allen, Chairman of the Science Research Council (largest of the five research councils).

The feeling has been growing among the research councils that cut-backs in the UGC grant have fallen disproportionately on science, engineering, and medicine which through their laboratories have greater service requirements than the humanities. Applications to the research councils are increasingly including requests for equipment which would normally have been purchased through the university, and demands are increasing on research council central facilities (such as computers).

The working party will collect evidence, Sir Alec told *Nature* last week "and we can't rely on just a few scraps of information". On the other hand, the problem was too urgent to wait for the drafting and processing of a large questionnaire. The party "will not look at the total level of academic manpower" but will consider the problem of post-doctoral fellows and the provision of technicians.

"There is broad agreement that something is going wrong" with dual support

said Sir Alec. "We will have to balance saying something quickly with saying something reliable." The party hopes to report by the end of the year.

Meanwhile budgets for the research councils and the universities have fallen in real terms since 1978-9. Last week the UGC grant for the academic year 1980-1 was announced at £987 million, which says the Department of Education and Science "taking into account . . . the withdrawal of the subsidy for new overseas students . . . is about 2% lower in real terms than in 1979-80". The ABRC budget was also announced at £383 million for 1980-81 (see *Nature*, 3 April, page 000) with small increases for subsequent years, but these values are lower than those projected by the previous Labour government.

Jobs are also under pressure from these cuts, leading to a rapidly increasing average age for research groups; so it is hoped in some quarters that the terms of reference of the Merrison party will allow it to consider people as well as equipment.

● **EEC students to pay less:** The demand that foreign students must pay full tuition fees at British universities has been relaxed

for members of European Community countries, according to a report in *The Times*. This will increase pressure from Commonwealth countries (which account for half the UK's foreign students) that they also should get special treatment. The government is probably relying on the small number of EEC students in Britain remaining small, but, says *The Times*, it may have neglected the 3,500 Greek students who already outnumber EEC students. From 1981 Greece will be a member of the EEC and there may be an even greater inflow of students from the country.

● **£5 million for overseas student problem:** Mark Carlisle, Secretary of State for Education and Science, announced in the House of Commons last week that £5 million would be available in the academic year 1980-81 "to help ensure that uncertainty about prospective income from overseas students does not adversely affect selected postgraduate work of particular importance to this country". University sources last week had had no official indication of the meaning of this phrase.

Robert Walgate

Soviet Union

Media accuses US of war-mongering

US allegations about a bacteriological warfare incident at Sverdlovsk were met by the Soviet Union not merely with protests that the US was attempting to wreck the Geneva review conference on bacteriological warfare, but also by counter-allegations that the US was preparing itself for chemical war. In a major media campaign, the Soviet Union has cited sources ranging from *Newsweek* to "documents of the Church of Scientology" to support claims that the US is amassing "wet-eye" (nerve gas) and "dry eye" (stable toxic agent) bombs, carrying out experiments with toxic chemicals on US citizens, building up twelve arsenals of nerve gas, bacteriological aerosols, narcotics, defoliants and herbicides, while working on a "new generation" of binary gas chemical weapon "causing death in a matter of split seconds".

Much of the radio material, in both Russian and English, was beamed at the Third World. Special emphasis was given to alleged CIA experiments on "coloured people" in order to test the vulnerability of different ethnic groups. And a whooping cough epidemic of the mid-1960s in Florida, which resulted in 12 deaths, was specifically attributed to the CIA.

Nor was Britain exempt from such charges — it was attacked for alleged work on bubonic plague virus (citing the *Daily Telegraph*, training in the use of toxins at the army staff college (a "recent British TV film") and the new firing range near Porton Down (*Now!*), "officially, being used to devise protection against chemical warfare").

A few days after Tass's accusation, in the "Russian for abroad" service of Moscow radio, that the US are working, in "top-secret military laboratories", to "cultivate bacteria which could cause mass epidemics like anthrax, typhoid, plague, smallpox, etc", it had to elaborate on the official explanation of the Sverdlovsk incident. It said it was an outbreak of anthrax, caused by "adverse weather conditions in the autumn-winter of 1978-79" (which made sheep and cattle susceptible to contagious diseases), lack of personal hygiene in tending livestock, and the purchase of unbranded meat, wool and hides from unauthorized individuals. In spite of the struggle against the disease, it was explained, anthrax has never been completely eradicated from the Urals — thereby unfortunately providing a weapon for the US hawks to "call into validity [the bacteriological weapons] convention" and to "whip up the arms race".

Vera Rich

Soviet Union

Academy stresses applied science

LAST November, Mr Brezhnev, in a major speech, charged the Soviet Academy of Sciences with greater responsibility for applied science and technology. Last month's annual general meeting of the Academy stressed that this new emphasis is well to the fore in its thinking.

How far this reflects a genuine change of direction is, of course, disputable. The Academy has already made it clear that it had been involved in applied research for some time before Brezhnev's speech. Nevertheless, the emphasis in this year's reports on the applied value of the Academy's work may be significant. President of the Academy Anatolii Aleksandrov, in his Presidential address, stressed particularly the work of the Shumyakin Institute of Bio-organic Chemistry, not only on protein structure, but also on genetic engineering, with, he implied, potential economic applications. He likewise commended the Academy's Institute of Microorganisms Biochemistry and Physiology for its work on artificial proteins for animal feed, based on oil products and natural gas, and in particular, the organic chemistry data bank established at Novosibirsk, which, he said, would enable scientists to develop new organic compounds with predetermined properties.

Physics and Geography Department of the Academy, Leonid Brekhovskikh outlined possible uses for the Soviet Union's glaciers and ice cover. His department, he said, had

devoted much time and resources to the study of glaciers as a fresh water source, since they contained "twice as much fresh water" as all the world's rivers and lakes. Ice could also, he said, be used as a building material in northern areas, when constructing port berths and laying roads.

From the newly exploited hinterland of the Baikal-Amur mainline railway, Academician Aleksandr Yanshin reported that after many years of forecasting and prospecting for potassium salts in Siberia, a major deposit, sufficient to ensure the "chemicalisation of agriculture in Siberia" had been located in the north of the Irkutsk oblast'. While Academician Aleksandr Fokin, Deputy Chief Academic Secretary to the Presidium, in effect summarised the tone of the meeting by stating that great attention was being paid to links between research and production.

To carry out this massive programme, the meeting was told, Soviet funding for science had been raised by 55% during the past seven years (while the number of scientists actively involved in research had grown by 50% to 1,300,000).

In his Presidential address, Aleksandrov also stressed that, to solve such global problems as the search for new energy sources and the exploration of space, international cooperation was essential. He censured the US administration for curtailing Soviet-American scientific ties and exchanges.

Vera Rich

NEWS IN BRIEF

US produces draft climate plan

RESEARCH into the impact on the environment and society of increased carbon dioxide levels in the atmosphere, and into the effects of climatic change on world food production, are two of the top priorities that have been selected for attention under the draft of a National Climate Plan, published in Washington last week.

The plan presents a five-year programme for climate research, as required by the National Climate Programme Act of 1978. The carbon dioxide study has been selected as a "principal thrust" by the Department of Energy and the Department of Agriculture.

Also under the proposed climate plan, the National Aeronautics and Space Administration will give priority to efforts at understanding how the climate system gains and loses radiant energy. And the National Science Foundation will lead a major coordinated effort to understand the oceans' role in climate, in particular with heat-flux experiments planned in the Atlantic Ocean, perhaps leading to "a series of major international experiments in the late 1980s and early 1990s". The climate programme will coordinate the research efforts of all federal agencies, with the National Oceanic and Atmospheric Administration (NOAA) acting as the lead agency. NOAA's principal thrusts will be in the generation and dissemination of climate information and climate prediction.

US agency denies Agent Orange cover up

OFFICIALS of the Veterans Administration denied congressional charges last week that an official of the administration had referred to the herbicide 2,4,5-T as carcinogenic and mutagenic in a memorandum written in 1977. The charge had been made by two congressmen last month, who had obtained a copy of a memorandum with 'US government' written across the top, which said that the herbicide, known as Agent Orange, had been shown to "intercept the genetic DNA message process to an unborn fetus, thereby resulting in deformed children being born".

In producing the memorandum, the congressmen accused the administration of giving misleading information when it had earlier testified that there was no evidence linking the herbicide with human health problems. However, in a reply, Mr Max Cleland, administrator of the Veterans Administration, said that the VA official, whose telephone conversation was supposed to be the source of the memoran-

dum, had pointed out that it was "inaccurate in numerous points". Mr Cleland said that no-one in the VA had seen the memo before, and that it had been impossible to identify the author.

Australia orders Agent Orange inquiry

THE Australian government announced last week its intention to study the effects of Agent Orange, the dioxin containing herbicide used by the US to defoliate the countryside in Vietnam. Following a campaign by community and servicemen's groups, the government has granted \$2 million to survey the health of 41,000 Australian servicemen who were exposed to the chemical during the Vietnam war. In addition, the health of the families of the exposed servicemen will be studied. The study will be carried out by the Commonwealth Institute of Health of Sydney University. Veterans organisations have criticised the proposal saying that the survey will take too long to complete to prevent injury to those exposed. Mr K.G. Schultz, national secretary of the Returned Services League, said that a smaller sample would provide sufficient information about ill effects faster than the full survey. The Vietnam Veterans Action Association said it already had an overwhelming case for compensation with cases of birth defects that included missing limbs, club feet and cleft palates.

UK minister welcomes ASTMS cancer document

THE UK Secretary of State for Employment stated in parliament that he welcomed the Association of Scientific, Technical and Managerial Staffs document on occupational carcinogens (*Nature*, 20 March, page 203). In a written response to a parliamentary question by Renee Short asking if the government will take action "as a matter of urgency" on the document the minister said: "I welcome the initiative this union has taken in preparing the document, which I understand is to provide safety representatives and union officers with information. I propose to await advice from the chairman of the Health and Safety Executive before deciding whether discussions with the HSE or other interested parties are necessary".

UKAEA agrees PWR cracks a hazard

In a supplementary memorandum to the select committee on energy, the UK Atomic Energy Authority has agreed that the possibility of pressure vessel cracks

constitutes an insufficiently explored hazard of pressurised water reactors according to a report in *The Times*. The memorandum agrees with the analysis of Sir Alan Cottrell (*Nature*, 28 February page 803) that cracks as long as one inch could develop without detection in the pressure vessel which could lead to a rapid uncontrollable break resulting in a complete loss of coolant accident. Development of more advanced detection techniques than were previously thought necessary is required before the American design PWRs can be used in the UK. "It is vital that the necessary development work be undertaken as a matter of urgency" the memorandum is reported as saying.

In the meantime, Britain's ten year plan to introduce PWRs starting in 1982 may be delayed for another reason. The Nuclear Installations Inspectorate told the select committee last week that the Central Electricity Generating Board had yet to present its PWR design for safety clearance and to conclude a licensing agreement with Westinghouse. The delay, senior NII officials said, meant that the necessary safety studies could not be completed by the 1982 deadline.

In related developments, the Prime Minister is withholding approval for the construction of two scheduled advanced gas cooled reactors. The Prime Minister is a strong advocate of the PWR and has ordered further studies of the AGR proposal.

● Mr David Steel, leader of the Liberal Party, criticised Mrs Thatcher's "nuclear obsession" at the UK's largest ever anti-nuclear rally last week held on the anniversary of the Harrisburg accident. Fifteen thousand demonstrators from many British anti-nuclear groups attended.

Debendox safe, says UK committee

THE UK committee on the Safety of Medicines has found no evidence that the drug Debendox, an anti-nausea drug used by pregnant women, causes birth defects. The committee completed its third review of the drug at the end of last month. Two previous reviews in 1978 and 1979 had also found that the drug was safe.

Concern about Debendox has been stimulated by the recent court case in the US — where the drug is manufactured under the name Bendectin — which found that it had caused congenital abnormalities in a boy. The UK Minister of Health asked the committee to review its safety.

The committee has said that the level of abnormalities in the children of the 3,500,000 women who have taken the drug in the UK over the past 20 years, is no more than in the population as a whole.

NEWS AND VIEW

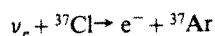
Is the Sun still shining?

from F.E. Close

THE conjunction of cosmology and particle physics as the result of recent developments in high energy physics formed the subject of a Wolfson Invitation Lecture given at the University of Oxford by J. Ellis of CERN. Taking as his title 'The very large and the very small' one of his themes was the current interest in neutrinos and how an understanding of their physics can provide explanations for some apparent anomalies in the Sun's interior.

Neutrinos are ghostly particles that carry no electrical charge and interact visibly only by means of the weak force. They are produced in stars, like the Sun, when light nuclei fuse to form heavy nuclei with consequent release of energy. They stream out from the Sun and can be detected on Earth by monitoring their reactions with atomic nuclei. In particular, their reaction with chlorine, which is readily available in ordinary cleaning fluids is used to calculate the incident neutrino flux.

When neutrinos interact with the ^{37}Cl nuclei in large underground tanks of cleaning fluid they initiate the reaction



by a neutron-proton conversion. The argon produced is extracted by helium purging, separated from the helium mass and finally introduced into a small proportional counter. Argon-37 undergoes K-capture decay with a 35 day period. Almost all these K capture events are detected and so the amount of argon is inferred and in turn the incident neutrino flux responsible for creating it in the first place can be calculated. This quantity is expressed in solar neutrino units (SNU). But although theoretical understanding of the Sun's interior requires that about 6 ± 2

SNU be observed, experiment has detected only a fraction of this. (A meeting in Rome during early February gave 2.2 ± 0.3 as the most up-to-date result).

One possibility is that our theoretical understanding of the Sun is badly wrong somehow. The next source of blame is in the inferences drawn from the experimental rate of detection.

Detection exploiting chlorine nuclei has several attractive features, not least of which is the abundant availability of cleaning fluid. However, a great disadvantage is that the transition can only be triggered by neutrinos that have at least 0.8 MeV energy. This is far above the maximum energy of neutrinos produced in $p + p \rightarrow d + e^- + \nu$ which is the main thermonuclear reaction in the Sun, and so the detection technique is insensitive to this important flux of neutrinos.

These low-energy neutrinos could be picked up through the nuclear transition from gallium-71 to germanium-71, which can be initiated by neutrinos with energies as low as 0.23 MeV. But to gain such information a target containing several tons of gallium would be required which is larger than the annual world production. Even were this region of the spectrum better studied however, it is thought unlikely that all the discrepancy with theory would be removed.

Another possibility is that the Sun has stopped shining. If the thermonuclear processes in the heart of the Sun have run down, there will be a significant drop in the neutrinos produced and a low flux will be detected on Earth. This is because the Sun is almost transparent to neutrinos and so they stream out from the core unhindered. In contrast, the Sun is quite opaque to photons and those that irradiate the Earth have come from the Sun's outer reaches. It would be some hundreds of thousands of

years before news of the changed circumstances in the Sun's core reached its surface. Thus we could be somewhere in this period, waiting for the surface photons to be turned off — the low neutrino flux is our advance warning of a real energy crisis.

Fortunately there is another possible cause for the low flux which is of particular interest in light of recent discoveries in particle physics.

Before 1976 two varieties of neutrino were known — one related to the electron (called electron-neutrino or ν_e), and one partnering the muon (muon-neutrino, ν_μ). Their masses are known to be orders of magnitude smaller than the lightest known particle (electron) and so it has been supposed that the neutrinos are massless. Since 1976 a third variety of neutrino has been discovered associated with the heavy lepton tau (τ). This ν_τ is less well studied but is also often referred to as 'massless'.

Masslessness and conservation principles are intimately linked, for example, the conservation of electrical charge is related to the masslessness of the photon. There is no conservation principle known that would require the neutrinos to be massless and so the possible consequences of their having masses have been studied.

If neutrinos have masses then the mass eigenstates could be linear superpositions of the ν_e, ν_μ, ν_τ eigenstates. Thus an electron-neutrino emitted in the Sun would be a linear superposition of three different mass eigenstates, each of which will oscillate with slightly different frequencies during the journey across space. By arrival at Earth a different linear superposition of mass eigenstates will be present which is equivalent to saying that what started out as ν_e is now a mixture of ν_e, ν_μ and ν_τ . If the mass differences are a few electron volts then the oscillations could be sufficiently rapid that the intensity of ν_e

arriving at Earth could be as low as 1/3 that which set out (the 1/3 being due to the three possibilities ν_e, ν_μ, ν_τ). This would naturally fit with the observed solar neutrino flux which is about 1/3 of that expected in the absence of oscillations.

The solar neutrinos produced in radioactive decays have a low energy. Higher energy neutrinos such as those produced in particle accelerators will have correspondingly larger oscillation lengths. As neutrinos can pass through the Earth without attenuation one could put detectors in line with a neutrino beam at CERN and see if the number of ν_μ , say, varies with distance.

One interesting theoretical development is the idea that all nature's forces could be unified at very high energies. Some versions of these theories predict that neutrinos have masses inversely propor-

tional to the unification scale. The unification is believed to occur at the order of the Planck mass (10^{19} GeV). This is large but not infinite and so the neutrino masses are small but not zero, of order 10^{-5} electron volts. If neutrino masses are indeed of this order then oscillation effects could be directly observable. In particular, the eccentricity of the Earth's orbit gives a natural variation to the distance that neutrinos have travelled *en route* from the Sun. Neutrino masses of the order of 10^{-5} eV could account for the solar neutrino anomaly, and provide an annual periodic variation in intensity of electron neutrinos which could be looked for. Such an observation would be exciting in its own right, would indirectly support attempts to unify the natural forces and would indeed resolve the solar neutrino anomaly, avoiding the catastrophic alternative. □

conditions produce new outer membrane proteins which act as receptors for Fe^{3+} -enterochelin (McIntosh & Earhart *Biochem.* **81**, 749; 1977). These organisms also contain altered species of tRNAs (Griffiths & Humphreys *Eur. J. Biochem.* **82**, 503; 1978). The same tRNA changes are found in *E. coli* recovered from the peritoneal cavities of lethally infected animals (Griffiths *et al.* *Infect. Immun.* **22**, 312; 1978). The alterations, which arise from an undermodification of the hypermodified nucleoside 2-methylthio- N^6 -(Δ^2 isopentenyl) adenosine in the tRNAs during growth under iron restricted conditions, may play an important part in regulating the expression of operons of the aromatic amino acid biosynthetic pathway from which enterochelin is synthesized by way of a branch pathway (Griffiths & Buck *Soc. Gen. Microbiol. Quart.* **7**, 10; 1979).

In a recent issue of *Infection and Immunity* (26, 925; 1979) P. Williams shows that *E. coli* carrying colicin V (Col V) plasmids possess another iron-sequestering mechanism, in addition to the enterochelin system, and suggests that this may be a contributory factor in the virulence of invasive strains. A well established link exists between Col V plasmids and the invasiveness of pathogenic *E. coli*. Most *E. coli* strains isolated from cases of bacteraemia in humans and domestic animals harbour Col V plasmids. Although the possession of a Col V plasmid markedly enhances virulence, colicin V activity itself is not essential (Binns *et al.* *Nature* **279**, 778; 1979; Quakenbush & Falkow *Infect. Immun.* **24**, 562; 1979). Williams finds that the possession of the Col V plasmid usually allows the bacteria to grow significantly faster in the presence of transferrin. However, this is not always the case. One Col V plasmid, which did not confer any growth advantage in conditions of iron stress, has been shown by Binns *et al.* to carry determinants for resistance to the bactericidal effects of serum. The acquisition of either characteristic, serum resistance or an enhanced ability to grow under iron stress, could confer a selective advantage on *E. coli* *in vivo*. The acquisition of both determinants would give an organism an even better advantage. It remains to be seen whether or not the plasmid in *V. anguillarum* mediates other virulence determinants in addition to enhancing the ability of the bacteria to grow in iron-restricted conditions.

The presence of a plasmid is not, of course, always required for virulence, nor is it the only factor involved. A further complication in the case of Gram negative pathogens is the natural variation in the O and K antigens of the bacterial cell wall. There is undoubtedly a connection between the structure of these complex polysaccharides, which determine serological specificity, and the virulence of the organism (Orskov *et al.* *Bact. Rev.* **41**, 667, 1977; Merson *et al.* *Infect. Immun.* **23**, 325;

Iron, plasmids and infection

from E. Griffiths, Henry J. Rogers & J.J. Bullen

AN important development in our understanding of bacterial infections has come from the realization that genetic determinants for certain virulence characteristics, such as haemolysins, toxins and adhesive factors, can be carried by plasmids. In this issue of *Nature* (page 566), J.H. Crosa reports on the presence of a plasmid in highly virulent strains of the fish pathogen *Vibrio anguillarum* which mediates a novel virulence mechanism, that of an efficient iron sequestering system. On losing the plasmid, *V. anguillarum* loses its virulence and also its ability to grow under conditions of iron restriction imposed by the serum iron binding protein, transferrin.

Several years ago it was found that iron compounds could abolish the antibacterial effects of body fluids *in vitro* and enhance bacterial virulence *in vivo*. These studies have led to an increased knowledge of the mechanisms of resistance to bacterial infection and have also provided an explanation for the changes in resistance which often accompany alterations in the iron status of the host (Bullen *et al.* *Curr. Top. Microbiol. Immun.* **80**, 1; 1978; Weinberg *Microbiol. Rev.* **42**, 45; 1978). We now know that although there is plenty of iron present in the body fluids of vertebrates, the iron content of human plasma for example is 20 μM , the amount of free iron is of the order of 10^{-18}M , which is far too low for bacterial growth. This extremely low concentration of free

iron is due to the presence of iron binding proteins, transferrin in blood and lymph and lactoferrin in secretions, with which the ferric ion is very strongly associated. Pathogenic organisms which multiply successfully under these conditions must possess mechanisms for assimilating protein bound iron or acquiring it from liberated haem.

Several organisms are known to produce iron chelating agents. *Vibrio cholerae*, for example, produces a phenolate iron chelator (Payne & Finkelstein *Infect. Immun.* **20**, 310; 1978) and *Pseudomonas aeruginosa*, iron chelators of both the phenolate and hydroxamate type (Liu & Shokrani *Infect. Immun.* **22**, 878; 1978). The best studied system, however, is that in bacteria of the genera *Salmonella*, *Escherichia* and *Klebsiella* which secrete the iron chelating compound enterochelin under iron restriction *in vitro*. This compound, which is the cyclic trimer of 2,3,-dihydroxybenzoyl serine, removes iron from iron-binding proteins and it has been proposed that its secretion is essential for growth of the organisms *in vivo* (Rogers *Infect. Immun.* **7**, 445; 1973). Recently, Griffiths and Humphreys (*Infect. Immun.*, in the press) have shown that enterochelin is produced *in vivo* during fatal infections with *E. coli*. Yancey *et al.* (*Infect. Immun.* **24**, 174; 1979) have reported that the loss by mutation of the ability to synthesize enterochelin greatly reduces the virulence of *Salmonella typhimurium* and also inhibits its ability to grow in human serum. In addition to secreting enterochelin, *E. coli* growing under iron-restricted

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1979). There are *E. coli* strains for example which belong to classically recognized enteropathogenic serotypes that are non-toxicogenic and non-invasive according to the usual criteria, and do not seem to harbour virulence plasmids but which nevertheless do cause severe disease (Levine *et al. Lancet* **I**, 1119; 1978; Scotland *et al. FEMS Microbiol. Lett.* **7**, 15; 1980; Ulshen & Rollo *New Engl. J. Med.* **302**, 99; 1980). In an era when resistance to known antibiotics seems to be increasing we clearly need to know far more about the mechanisms of microbial pathogenicity and host resistance. Although good progress is now being made, it is more than likely that the versatile bacterium still has many more surprises in store for us. □

X-ray astronomy with the Einstein observatory

from J.L. Culhane

NASA's Einstein X-ray observatory satellite was launched in November 1978. It carried a large reflecting X-ray telescope, of a few arc seconds angular resolution, equipped with various focal plane detectors for X-ray imaging and spectroscopy. Before its launching, X-ray astronomy had made spectacular advances through observations undertaken with satellites instrumented by US, UK and Netherlands research groups. These missions established the existence of several hundred X-ray sources and discovered a range of exciting phenomena that included the presence of rotating neutron stars and perhaps even black holes in binary star systems, the existence of high temperature gas spread throughout clusters of galaxies, the identification of Seyfert galaxies as X-ray sources and the heating of interstellar plasma to million degree temperatures by the shock waves that expand from supernova explosions. However with the exception of the small X-ray reflectors on the Copernicus satellite, and the modulation collimators on SAS-3, Ariel V and HEAO-1, all of these missions used collimated proportional counter detectors with fields of view of several square degrees. Thus it was to be expected that the availability of a truly imaging telescope with arc second resolution would bring about a transformation in our knowledge of the X-ray sky.

A preliminary measure of this transformation is provided in a recent issue of *Astrophysical Journal Letters* (234, L1-L81; 1979) which contains 15 papers devoted to the first results obtained with

the Einstein satellite. The observing programme included many specific objects but, because of the two orders of magnitude improvement in sensitivity over previous instruments, it was also decided to use the telescope for deep surveys of several regions of the sky that contained no previously known X-ray sources. Regions in Draco and in Eridanus have been studied and 43 new sources identified. Ten of these are believed to be extragalactic, the majority probably quasars and on the basis of an extrapolation to lower fluxes, the contribution of such sources to the diffuse X-ray background is established as almost 40%. If the majority of these sources were indeed quasars and active galaxies, then on the basis of our current understanding of X-ray to optical luminosity ratios, quasars brighter than $B \sim 20.3$ mag could account for essentially all the X-ray background. If confirmed by future observations, this could prove to be one of the most significant discoveries made by Einstein. Quasars are in fact established as a class of luminous X-ray emitters by the detection of more than 30 where only 3 X-ray quasars were known before the Einstein mission. Many more are being found as observations continue.

It has been known for some time that the giant radio galaxy, Centaurus A, has an X-ray source located in the nucleus of the associated active galaxy. Observations with the Einstein telescopes have now established that in addition to the point X-ray source coincident with the infrared nucleus, a diffuse component associated with the inner radio lobes, an emitting region of about 4 arc min extent surrounding the nucleus and an X-ray jet lying between the nucleus and the NE inner radio lobe are also present. The paper which reports these discoveries suggests that the radio lobe component may arise from the inverse Compton scattering of the microwave background while the existence of a jet provides further evidence of the continuing supply of energy to the lobes from the nucleus. This observation of X-ray structure around the nucleus of the best studied giant radio galaxy could be of the utmost importance in helping us understand the energy supply mechanism for radio galaxies in general.

The extended sources of X-ray emission associated with clusters of galaxies have naturally been objects of much interest to the Einstein observers. Several papers are devoted to results on the structure and evolution of these sources. However one of the most striking observations has been the detection, from the neighbourhood of M87 in the Virgo cluster, of an emission line characteristic of ionized oxygen (hydrogen-like O^{+7}) by the focal plane crystal spectrometer. The detection of highly ionized iron (Fe^{+24}) emission lines by the Ariel V and OSO-8 satellites in the X-ray spectra of several clusters had previously established the presence of high temperature ($30 \times 10^6 \text{ K} < T < 120 \times 10^6$

K) plasma in these sources. However the detection of material around individual galaxies at temperatures less than 10^7 K has important implications for theories of gas infall and cooling in clusters. More recent observations with both the crystal and solid state spectrometers have indicated a similar situation near NGC 1275 in the Perseus cluster and we await further observations of the gas near massive galaxies in other clusters with considerable interest.

The Uhuru and Ariel V surveys indicated the presence of many X-ray sources in the plane of our galaxy thus suggesting that similar objects should exist in other normal galaxies. A dramatic measure of the advance due to Einstein is provided by the detection of 69 discrete and 7 diffuse sources in the nearby spiral galaxy M31. This is all the more striking when we recall that the Uhuru catalogue listed M31 as a single X-ray source with an intensity that was only just detectable. Thus the enormous power of a high resolution imaging telescope is clearly demonstrated. Similar advances have been made in observations within the galaxy. A group of six point sources has been found in the neighbourhood of Cygnus X-3 where previously only the latter was known to exist while the existence of more than 20 sources has been established in the Orion nebula. Work in progress at the Harvard Centre for Astrophysics which has been reported at a recent conference in Boston although not presented in the Einstein issue of *Astrophysical Journal*, suggests that X-ray emission has been detected from essentially all of the star classes represented in the H-R diagram. Thus X-ray astronomy has in less than two decades advanced from a concern with a small number of unusually energetic objects to occupy a position of central relevance for the entire field of astrophysics.

While the discovery of X-ray emission from a large number of stars is of considerable importance, a hint of the real potential of X-ray astronomy is provided by the beautiful emission line spectrum of the Capella system that has been obtained with the solid state spectrometer. X-ray emission lines from Mg, Si, S and Fe are observed and it is at once clear that the deployment of high resolution X-ray spectrometers will allow us to apply the sophisticated plasma diagnostic techniques, that have been refined over many years in observations of the solar corona and of laboratory plasmas, to the study of the detailed physics of a wide range of objects. The importance of X-ray spectroscopy is further emphasized by the X-ray emission line spectrum of the supernova remnant Cassiopeia A, which when compared with the detailed map of the remnant obtained with the imaging detectors, permits a considerable increase in our understanding of these objects.

Although the Einstein observatory has carried out a series of truly staggering observations in its first year of operation,

one cannot help but feel a slight disappointment that as yet nothing has emerged to rival in excitement the discovery of X-ray emitting binary systems and clusters of galaxies by the Uhuru satellite. So far no faint new categories of X-ray objects have been discovered. However to complain is merely to emphasize the extraordinarily high standards that have been set in the subject from the beginning. We are witnessing the establishment of a mature astronomical discipline which, along with optical and radio astronomy, must from now on have an essential role in our study of the Universe. The manner in which the Einstein mission is bringing about this maturity will surely come to be regarded as one of the substantial achievements of twentieth century astronomy. □

Exploiting papyrus

from Peter D. Moore

THE potential of papyrus (*Cyperus papyrus*) to 'clean up' tropical lakes in danger of eutrophication (see *News & Views* 276, 560; 1978) is again under review. In particular work by Gaudet (University of Nairobi) on Lake Naivasha in Kenya provides grounds for optimism.

Papyrus forms floating swamps in the peripheral zones of tropical lakes especially where incoming rivers debouch their waters (Gaudet *Aquatic Bot.* 3, 1; 1977). The plant is robust (up to 5 m tall) and productive, so considerable quantities of nutrient ions are absorbed during swamp growth. Gaudet (*Ecology* 58, 415; 1977) made some preliminary estimates of nutrient uptake based on certain fairly simple assumptions concerning the rates of culm replacement within a papyrus stand. He concluded that rates of uptake could amount to N, 62 kg m⁻²; P, 5 kg m⁻²; K, 103 kg m⁻² during one complete turnover of culm replacement (147 days). Nutrient losses would occur at the same time as a result of death, decay and leaching of older culms. Losses were considered to amount to two thirds of the material absorbed, the remainder accumulating within the swamp peat which descends from the floating mat to accrete with the other sediments below.

When inflow is high, water will move faster below the floating raft and will dilute the ionic concentration in this zone. On the other hand, in dry periods the concentration beneath the raft will rise as a result of evaporation. Fast through-flow could, of course, reduce the efficiency of the swamp in modifying water quality. Gaudet has monitored these effects through the year at Lake Naivasha (*Verh. Int. Verien Limnol.* 20, 2202; 1978) and has indeed shown such seasonal variations.

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The overall nutrient trapping efficiency of the swamp, however, was low as a result of the rapid water movement in wet periods. The final retention of nutrients in the sediments below the mat was of the order of 10% for magnesium, ranging down to 1% for calcium. So overall, the swamp is not greatly influencing water quality.

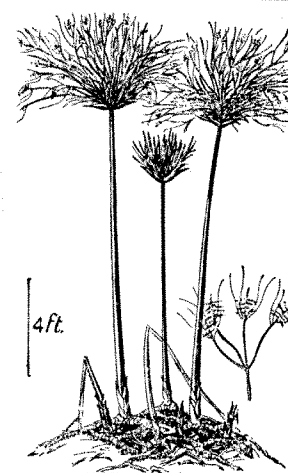
These figures may not fully reflect the precise operation of the process, however, because of the particular analytical techniques used. All water samples were filtered before chemical analysis, which would have removed organic particulate matter. Gaudet has now published additional data (*J. Ecol.* 67, 953; 1979) based on the use of sediment traps, and has expressed these as a percentage of annual input retained in the sediment. On this basis, the removal of elements is of the order P, 57%, S, 59%; Fe, 83%, Mn, 94%. So the swamp is acting as an efficient nutrient extractor, but much of the extracted material is being transported as detritus by the flow-through below the mat.

Nitrogen is an important exception, for there is a high degree of net fixation within the swamp system. Net gains within the ecosystem suggest fixation rates in excess of 50 g m⁻² annum⁻¹.

The efficiency of papyrus swamps as nutrient extractors would be increased if they were harvested periodically and the biomass removed. Thompson (in *The Nile, biology of an ancient river* (ed. J. Rzoska) 177, Junk, The Hague, 1976) has found that a stand of papyrus contains a mixed population of culms of various ages, with the life cycle of a culm taking about 150 days. Gaudet has shown that rapid accumulation of nutrients occurs in the early stages of growth; thus frequent cropping should result in a maximum nutrient drain on the system.

Thompson, Shewry and Woolhouse (*Bot. J. Linn. Soc. Lon.* 38, 299; 1979) have now conducted a detailed analysis of the population structure and productivity of papyrus culms in swamps of the Upemba basin of Zaire and in Uganda. The total biomass of the system seemed to be climatically determined, being 2.9 kg m⁻² in Uganda (545 m altitude), 1.1 kg m⁻² in Zaire (~1000 m) and 3.4 kg m⁻² at Gaudet's Lake Naivasha, Kenya (1830 m). But there was not such a strong, nor such a consistent variation in productivity between these areas; figures ranged between 10 and 35 g m⁻² day⁻¹. Some temperate reedswamps, such as *Scirpus lacustris* in Germany (*Westlake Biol. Rev.* 38, 385; 1963) have been described which overlap the lower end of this range, but these figures do emphasize the high productivity of *Cyperus papyrus*, which is considered by Thompson *et al.* to be a C₄ species.

So papyrus harvesting could provide a renewable energy resource for combustion at the same time as enhancing its efficiency in recovering nutrients from eutrophicated



Cyperus papyrus

waters. Work of the sort described here emphasizes the need to conserve tropical swamps until their full value can be assessed and until appropriate management regimes can be developed on the basis of controlled experimentation. Their value for local human populations could be considerable and their exploitation could relieve pressure on other, less easily renewed, ecosystems. □

Atomic hydrogen stabilized

from P. V. E. McClintock

SILVERA and Walraven's report (*Phys. Rev. Lett.* 44, 164; 1980), that they have succeeded in stabilizing bulk atomic hydrogen, suggests that the preparation of superfluid hydrogen may become a practical proposition in the not too distant future.

Superfluids, possessing the remarkable facility of frictionless flow behaviour, devoid of the viscous effects which characterize familiar liquids such as water, are exceedingly rare. The only examples known at the moment are the liquid heliums (⁴He below 2 K; ³He below 2 mK) and, in a slightly different sense, the electron gas in a superconducting metal. Thus if hydrogen and also, perhaps, its isotopes deuterium and tritium could be prepared in a similar state it would, at a stroke, double the number of superfluids accessible to experimental investigation.

This exciting possibility rests on the fact that, although ordinary molecular hydrogen (H₂) forms a solid at low temperatures, and is thus unable to become superfluid, no such restriction applies in the case of atomic hydrogen (H). The forces between a pair of hydrogen atoms depend profoundly on whether their electron spins lie mutually parallel or

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antiparallel. In the latter case, there is a very strong force of attraction, rapidly leading to formation of an H_2 molecule. In the former case, on the other hand, the interatomic force is so weak that it is possible, at least in principle, to cool a dense assembly of H atoms to the very low temperatures where superfluidity may be expected to manifest itself, without solidification occurring at all. The main difficulty, of course, is that of getting atomic hydrogen into a stable spin-aligned state, given that it has a very strong natural inclination to minimize its energy by forming H_2 .

Although Silvera and Walraven have followed the conventional approach of attempting to stabilize the H gas by a combination of high magnetic field and low temperature, their apparatus incorporates a number of novel and imaginative features. The atomic hydrogen itself was produced in the usual way by means of a discharge tube at room temperature but, rather than allowing the H gas merely to diffuse into the stabilization cell, they pumped it in using a specially designed vapour diffusion pump which, using helium as the working medium, possessed a unique capability in being able to operate at 0.48 K. This device was an essential feature of the overall experimental design because, as the same authors demonstrate in the paper immediately following (Walraven & Silvera *Phys. Rev. Lett.* **44**, 168; 1980), the H gas would otherwise have had a strong tendency to leak out of the stabilization cell again.

The authors also found that it was essential to cover the walls of the cell with a very thin layer of helium-4. Because hydrogen is insoluble in liquid helium, this coating served to insulate the H atoms from contact with the walls which, if it had occurred, would have produced spin-flips rapidly followed by recombination to ordinary molecular hydrogen.

A special type of bolometer was developed to demonstrate the presence of the H gas confined within the cell. This exploited the heat of recombination which is generated when H_2 is formed: after a controlled burning off of its helium coating, the bolometer provided an active surface on which recombination could take place. An estimate of the initial H density could be made by noting the extent of the recombination heating which occurred when the bolometer was triggered in this way. It was found that, after being filled, the cell could be left a period of several minutes without any measurable loss of H at all, implying that, at least within the timescale of the experiment, their stabilization scheme had been completely successful. The greatest density of H achieved was estimated as being not less than 1.8×10^{14} atoms cm^{-3} ; but the authors hope to be able to improve on this figure by several orders of magnitude (a factor of 10^3 or 10^6 being needed before the superfluid

effects would be expected), once they start trying.

These experiments represent a most encouraging step forward. They have raised the status of superfluid hydrogen from little more than a scientific pipe dream to being a very real possibility within the next few years. □

Are abundant proteins less variable?

from Yvonne Edwards & David A. Hopkinson

THE analysis of enzymes using gel electrophoresis and specific activity stains has revealed extensive genetic diversity in man and other species. At least 25% of the genes which determine enzyme structure are variable and the average level of heterozygosity per gene locus detectable by conventional unidimensional electrophoresis is in the region of 7 to 10% in man and other vertebrates and somewhat higher in insects such as *Drosophila* (Harris *et al. Ann. Hum. Genet., Lond.* **36**, 9; 1972). However, recent studies on the same species using the O'Farrell bidimensional gel electrophoresis technique (*J. biol. Chem.* **259**, 4007; 1975) suggest that the average heterozygosity at gene loci determining the particular array of proteins discernible by this procedure is about 1 to 4%, substantially lower than expected from the enzyme surveys. Are the data from the uni and bidimensional electrophoresis compatible?

The bidimensional technique provides a method for the analysis of proteins which occur in greater abundance than most of the enzyme proteins since it relies on non-specific protein staining or autoradiography. The proteins are denatured and separated by isoelectric focusing in the first dimension according to charge and by SDS gel electrophoresis in the second dimension according to molecular size. A large number of gene products can be analyzed in a single experiment, but side-by-side comparison of the complex patterns of several hundred spots is difficult and the identification of individual variation is not as straightforward as in the unidimensional electrophoresis of enzymes. However, two recent studies (McConkey *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 6500; 1979; Walton *et al. J. biol. Chem.* **254**, 7951; 1979) on the polypeptides of human fibroblasts are noteworthy since they elegantly solved the problem of side-by-side comparison by using a double label autoradiographic technique which permits

the simultaneous analysis of 3H -labelled and ^{14}C -labelled proteins in the same gel. Between 300 and 400 polypeptide spots from human fibroblasts were examined and the average heterozygosity was found to be in the region of 1%. Estimates of average heterozygosity lower than expected from enzyme surveys were also found in bidimensional electrophoretic analyses of smaller samples of *Drosophila* (Leigh Brown *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 2381; 1979) and mouse polypeptides (Racine *et al. Nature* **283**, 855; 1980).

The reasons for the apparent discrepancy between the results obtained by the different techniques are not clear, but there are two major lines of argument worth exploring. Either the polypeptides accessible to analysis by the bidimensional procedure derive from a class of proteins with genetic properties distinct from those of soluble enzymes or this method is not as efficient at detecting variant proteins as conventional electrophoresis.

Bidimensional analysis selects those polypeptides present at highest concentrations, but this feature almost certainly does not account for the apparent low incidences of genetic variation. On the contrary, there is good evidence that the relative abundance of a protein and levels of heterozygosity at particular gene loci are not directly related, for example amongst human proteins present at high concentration haemoglobin, carbonic anhydrase II and several of the serum proteins show genetic polymorphism. And more recently, extensive genetic heterogeneity of the major storage protein of *Pisum*, demonstrated by both uni and bidimensional analysis, has been reported (Casey *Heredity* **43**, 265; 1980).

However, the array of proteins analyzed by the two procedures differ not only in their relative abundance, but also in their subcellular origin. In the bidimensional system whole cells, tissues or organisms are solubilized by denaturation, so that many of the most abundant proteins are derived from structural components such as membranes and are very likely to have complex subunit structures. It can be argued that vital protein-protein interactions and associations between protein and lipid/carbohydrate moieties such as occur in membranes, place constraint on the number of mutations which can occur. Furthermore, it is now recognized that multimeric proteins involved in polypeptide interactions show less genetic variation than monomeric proteins (Harris *et al. Proc. natn. Acad. Sci. U.S.A.* **74**, 698; 1977; Zouras *Nature* **262**, 227; 1976; Ward *Biochem. Genet.* **15**, 123; 1977) and it is possible that multimers may be over-represented in the array of proteins analyzed by the bidimensional procedure.

Considering the question of efficiency there is a considerable body of evidence which testifies to the discriminatory power

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of the bidimensional method. Milman *et al. Proc. natn. Acad. Sci. U.S.A.* **23**, 4589; 1976) detected a mutation for the enzyme hypoxanthine guanine phosphoribosyl transferase by analysis of HeLa cell extracts, Commings (*Nature* **277**, 28; 1979) has reported a polymorphism of human brain polypeptides, and genetic variants of transferrin, recognized by conventional electrophoresis were demonstrated after bidimensional analysis (Anderson *et al. Biochem. Biophys. Res. Commun.* **88**, 258; 1979).

In all cases, the variants detected by the bidimensional procedure can be attributed to mutational events leading to a change in charge on the protein molecule. Many of the variants detected by conventional electrophoresis are also attributed to amino acid substitutions leading to a change in net charge and there is good reason to think that the affected residues lie on the surface of the protein. However, this type of charge change variant is more readily detected after electrophoresis of the native protein than of the denatured polypeptides, since in denaturing conditions the frictional resistance of the molecules is greatly increased and the charge change less exposed. Nevertheless, it is interesting to note that Steinberg *et al. (Cell* **10**, 381; 1977) have demonstrated that progressive carbamylation of AMP dependent protein kinase leads to discrete shifts in *pI* detectable by the bidimensional procedure, which presumably represent single charge changes. However, one of the problems associated with detecting amino acid modifications by isoelectric focusing is that the efficiency of the resolution, as judged by the relative shift of *pI*, will not be the same over the whole gel, but will depend upon the position in the *pH* gradient and the proportional change in charge.

One way in which this problem could be overcome is by modification of the conditions for bidimensional electrophoresis which is commonly carried out in a broad *pH* gradient and at standard gel concentrations. So far the possibilities for varying these conditions have scarcely been explored and it seems likely that if greater flexibility is introduced then somewhat higher levels of heterozygosity might be revealed.

In contrast, the unidimensional gel electrophoresis method provides many opportunities for manipulating conditions such as *pH*, buffer ions, gel concentrations, solvent polarity, temperature and sample concentration. Effects due to the incorporation of affinity ligands in the gels and interactions with sulphhydryl reagents can also be explored. And it is quite a common experience that analysis of an enzyme in a range of electrophoretic conditions leads to the identification of additional variants and an increased estimate of heterozygosity. On the face of it the unidimensional electrophoretic methods primarily detect

charge change variation, but in practice the procedure almost certainly demonstrates a range of other types of variants which arise as a result of uncharged amino acid replacements. Such variants may be revealed

by differences in affinity of the allozymes for coenzymes, variable interaction with different buffer ions and altered electrophoretic mobility due to conformational distortion. □

Growth hormone: deletions in the protein and introns in the gene

from M. Wallis

MODERN high resolution fractionation methods can reveal that most protein preparations are made up of several different closely-related species. In the case of pituitary growth hormone, such microheterogeneity has a multiplicity of origins, and its detailed analysis has provided a wealth of information about the biochemistry and genetics of the protein. Thus, heterogeneity at the N-terminus of bovine growth hormone is now known to be a consequence of ambiguous processing of the precursor (pregrowth hormone) while heterogeneity at residue 127 reflects an allelic variation in the population of cows from which the hormone is prepared. In the case of human growth hormone various enzymically modified forms of the protein have been described, which may reflect enzymatic processing of the hormone *in vivo*.

Perhaps the most interesting of the many growth hormone variants, however, is a form isolated from human pituitaries and characterized by U.J. Lewis and his colleagues at the Scripps Clinic, La Jolla (*J. Biol. Chem.* **253**, 2679; 1978); this they have called 20K human growth hormone, since it has a molecular weight 1000-2000 less than normal human growth hormone (22K). The 20K variant is smaller because it lacks 15 amino acid residues found in the normal form of the hormone. The precise position of this deletion has now been shown (Lewis, Bonewald & Lewis, *Biochem. Biophys. Res. Commun.* **92**, 511; 1980) to include the entire sequence between positions 32 and 46 (inclusive) of the polypeptide chain; otherwise 20K is identical to the normal form of human growth hormone. The variant possesses growth-promoting activity similar to that of normal human growth hormone, but lacks the insulin-like activity normally associated with the hormone. It exists in all human pituitaries, comprising about 15% of the total growth hormone content.

How is this remarkable variant formed? A clue may be provided by recent work on the structure of the growth hormone gene by H.M. Goodman and his colleagues at the University of California, San Francisco (Fiddes *et al. Proc. natn. Acad. Sci. U.S.A.* **76**, 4294; 1979). They have shown that the gene is split and contains at least

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three intervening sequences (introns). One of these occurs between the codons for residues 31 and 32 in the mRNA — precisely the position of the start of the deletion in 20K. The end of the deleted sequence (between residues 46 and 47) does not correspond to the position of another intervening sequence, but it is noteworthy that the last two bases of the codon for residue 46 (glutamine) are AG (Martal *et al. Science* **205**, 602; 1979), a base sequence found almost universally at the 3' end of an intervening sequence in the primary RNA transcript of a gene (Crick *Science* **204**, 264; 1979). It is difficult to believe that this partial correspondence between a deleted sequence in the protein and an intervening sequence in the corresponding gene is a coincidence. It seems likely, therefore, either that 20K represents the product of a duplicated gene in which an intervening sequence has been lengthened to include part of a coding sequence (exon) or that the messenger RNA for 20K is produced from the same primary RNA transcript (and therefore the same gene) as normal human growth hormone, which would therefore be processed in two different ways. In the second case, a region of the primary RNA transcript that is a coding region for normal human growth hormone would be treated as part of an intervening sequence for the 20K variant, and different ways of processing a single primary RNA transcript would lead to production of two different proteins.

Whichever of the two explanations is correct, it is difficult to avoid the conclusion that the occurrence of an intervening sequence in a gene has allowed the formation of a markedly different protein variant. Gilbert (*Nature* **271**, 501; 1978) has proposed that the existence of intervening sequences may allow large changes in protein structure, and this may well be an example of such a change. It is interesting in this connection that the rate of evolution of human growth hormone is much greater than that of the non-primate hormones (Wallis *Biol. Rev.* **52**, 35; 1975). Whether the potential benefit of such large evolutionary jumps is in itself sufficient to explain the occurrence of intervening sequences, or whether other causes led to their origin and the evolutionary jumps are a consequence of their existence, remains to be seen. □

Caenorhabditis Genetics Center

A *Caenorhabditis* Genetics Center (CGC), sponsored by the National Institute on Aging, is being established at the University of Missouri. The CGC will be responsible for acquisition, banking, and distribution of *C. elegans* strains and reference strains of other *Caenorhabditis* species. Related services will include maintenance and annual distribution of the genetic map of *C. elegans*, coordination of genetic nomenclature, and maintenance and distribution of a bibliography of research publications.

The centre is the only one planned for *Caenorhabditis elegans*, an organism that has attracted attention in recent years as a research model for genetics, neurobiology, and developmental biology as well as aging.

The establishment of a *Caenorhabditis* Genetics Center (CGC) will promote the rapid and orderly accumulation and documentation of the genetic wealth of *C. elegans* for the benefit of all types of biomedical research on this organism. The genetics of *C. elegans* already enjoys important advantages over more traditional genetic systems. The ability to freeze stocks permits the reliable preservation of all known mutant types. The ancestry of virtually all strains is known so most stocks can be accurately described. A uniform system of genetic nomenclature has been instituted. Since most genetic data have been accumulated in only a few laboratories, the centralization of these data is feasible.

The specific goals of the CGS are (1) to establish a reliable and accessible genetic stock repository, including represent-

ative mutant alleles of all characterized genes as well as chromosome rearrangements, (2) to coordinate and publicize a uniform genetic nomenclature, (3) to coordinate and publicize the delineation of the genetic map, and (4) to develop a computer-based data storage and retrieval system which will handle bibliographic information and data used to generate the genetic map, as well as descriptive data on mutant strains.

The CGC strain collection will include at least one allele of each identified gene, all available chromosomal rearrangements, and available closely linked double mutants, as well as other strains useful for genetic mapping. Laboratories providing mutants of *C. elegans* will be requested to include such information as name of strain; names of contained mutation(s); mutagen used; whether the strain was backcrossed and, if so, how many times; genes affected by the mutation(s); map location(s) of mutation(s); and data used to determine map locations.

C. elegans strains will be available without cost to all qualified investigators pursuing genetic and/or related studies with *C. elegans*. The Center will not be fully operational until the Fall of 1980, but some services are available now. Inquiries should be addressed to: Dr. Margaret M. Swanson, Curator, or Dr. Donald L. Riddle, Director, *Caenorhabditis* Genetics Center, Division of Biological Sciences, Tucker Hall, University of Missouri, Columbia, Missouri 65211.

Though the types vary considerably, many of the contexts from which they come are of high status, being predominantly rich burials. In addition a number of objects combine iron with a precious metal, such as iron rings with a covering of sheet gold or an iron pin with a gold head. It is clear that iron was being increasingly used in societies with a highly developed competence in metallurgy, frequently but not exclusively in association with objects of high status. Analyses have so far been few, but many of these early iron objects have been considered meteoric on the grounds of their high nickel content. There has been some controversy over objects with a rather lower percentage of nickel, but there can be no doubt that throughout the period, and increasingly towards the later phases, terrestrial ores were also being exploited.

Similar evidence of an early origin for iron-working has also come from other areas. Some of the most exciting archaeological discoveries of recent years have been made at Ban Chang in north-east Thailand. In addition to surprisingly early occurrences of agriculture, irrigation and copper metallurgy, iron was in use by at least 1600 BC. As Gorman and Charoenwongsa (*Expedition 18*, 14; 1976) show, iron was used especially for the blades of bronze-handled weapons found in rich graves. In China, iron was similarly used in a composite bimetallic weapon in a rich grave of the Shang period at K'ao-Cheng, perhaps around 1200 BC. As Chang (*Archaeology of China*, 351; 1977) points out, there too the iron may be of meteoric origin since other comparable weapons, now in western museums, have been proved to be so.

In Europe too, reports of early iron have increased considerably in recent years. Brongers and Woltering (*Prehistorie van Nederland*, 97; 1978) describe finds of iron and slag with radiocarbon dates as early as the 12th century BC, while an iron fragment from a knife found in Slovakia is dated to 1465±35 BC (Butler in *IX Congress UISPP, Résumé*, 431; 1976). Another line of evidence has been followed by Bouzek, who has studied the technology of the decoration of bronzes in the late bronze age (*Zeitschr. f. Archäol.* 12, 9; 1978). He found that iron tools were increasingly used from 1100 BC, a conclusion now supported by the discovery of the appropriate iron tools in contemporary graves. The only detailed regional study is by Laszlo (*Acta Archaeol. Hung.* 29, 53; 1977), who documents recent discoveries in Romania, where iron became progressively more common through the later bronze age from 1200 BC onwards, copying the local bronze forms and even being manufactured in the same workshops as bronze.

All these regions share a number of common features such as the pre-existence of a developed metallurgical technology, the copying of bronze forms in iron, the large proportion of iron objects which are

The early development of iron-working

from T. C. Champion

TRADITIONAL explanations for the adoption of technological innovations in archaeology are being called into question by recent research. Much attention has been paid to the origins of metallurgy, and to the possibility of multiple independent inventions of copper working, but until recently the development of iron working had been relatively ignored. Recent work now suggests that the discovery of iron did not occur in one unique area from which its knowledge was diffused, that early iron was not superior to bronze, and that bronze-using communities did not adopt it immediately. Instead, what has usually been regarded as an obvious technological advance appears, when seen against the background of current economic and social conditions, to be due to much more complex reasons.

The traditional view held that the technology of iron was developed only in the Near East and was guarded as a royal

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monopoly by the Hittite empire during the second millennium. The collapse of the empire about 1200 BC allowed the knowledge of iron-working to spread to other areas where the new material was progressively adopted as its potential for making superior weapons and tools was appreciated. This view was based as much on the interpretation of Hittite historical documents as on archaeological finds but recent work by Waldbaum (*Stud. Mediterr. Archaeol.* 54, 1; 1978) now shows it to be untenable. Iron was known and used regularly throughout the area of the eastern Mediterranean from the early bronze age onwards and though some finds are known from as early as the beginning of the third millennium they become much more common in the mid-second millennium. Tools, weapons and jewelry are all made in iron. As the forms are in most cases specifically local ones which copy pre-existing bronze examples it would seem that iron was worked locally rather than imported from a single centre.

of high status, and in many cases the use of iron in combination with other metals. A rather different picture is presented in India, where Chakrabarti (*Antiquity* 50, 114; 1976) has argued for an independent growth of iron-working by 800 BC. Here, however, the range of iron goods is predominantly domestic, and a phase of gradual development has yet to be documented. It is possible that the earliest stages of iron-working in the subcontinent have yet to be explored as the date of 1025 \pm 110 BC reported by Jain (*Prehistory and Protohistory of India*, 191; 1979) for a layer containing small iron tools would support the suggestion of a more extended development. Even more problematical is the question of the origin of iron in west Africa. The technology described by Tylecote (*W. Afr. J. Archaeol.* 5, 1; 1975) from the Nok culture of Nigeria is already well developed by about the fourth century BC, too early to be derived from any of the known industries of the north-east in Egypt or the Sudan. The solution proposed is that the technology diffused from the north coast across the Sahara but the possibility of independent invention cannot be ruled out. There was no previous tradition of metallurgy but the ready availability of high-grade ores and the pyrotechnic skill demonstrated by the Nok clay figurines fulfil some of the necessary preconditions.

The theory of a unique invention of iron technology is clearly now very difficult, if not impossible, to maintain, and the focus of research has moved from questions of diffusion to the conditions under which the technology developed. Although the west African case still remains problematic, the other areas all shared a competent bronze metallurgy and in most of them the use of early iron is strongly associated with high status objects. The development of iron technology may therefore be seen as taking place in stratified societies where considerable technological resources are invested in producing prestige items. This stage of early development is quite distinct from a later phase when iron replaces bronze for a wide range of everyday items. Snodgrass (*Dark Age of Greece*, 213; 1971) argued, on the basis of limited analyses of early iron objects, that they were in fact not superior to the quality of bronzes but actually inferior and he attributed the wide-scale adoption of iron in Greece to an interruption in the supply of raw materials forcing the use of local resources. Maddin, Muhly and Wheeler (*Scient. Amer.* 237, 122; 1977) have described the discovery of the essential techniques of carburization, quenching and tempering in the Near East, but they too, like Waldbaum, ascribe the demise of bronze to a breakdown in the supply of raw materials, especially tin. Further analyses are needed of both iron and bronze objects, but it is now recognized that the problems of the discovery and adoption of iron can only be understood in relation to contemporary social and economic conditions. □

Ocean trench topography

from D.L. Turcotte

THE anomalous topography and negative gravity anomalies of ocean trenches are readily explained by plate tectonics. The oceanic lithosphere bends and descends into the interior of the Earth in a process referred to as subduction. This sounds relatively straightforward until one realises that the oceanic lithosphere is a 100 km thick plate made up of cool, brittle rock. How can a plate 100 km thick made up of a brittle material be bent through an angle of 45° or more in the subduction process?

Two recent papers examine alternative hypotheses for the bending behaviour of the lithosphere. Chapple and Forsyth (*J. geophys. Res.* 84, 6729; 1979) argue in favour of an elastic-perfectly plastic rheology. In the upper 20 km they assume that the rock is relatively weak due to low lithostatic pressure (the weight of the overlying rock) and postulate a yield stress of 1 kbar. In this region the tensional bending stresses due to the flexure of the lithosphere at an ocean trench give the normal faulting earthquakes observed seaward of many ocean trenches. Between depths of 20 and 50 km a yield stress of 6 kbar is postulated. At stresses less than 6 kbar the rock is assumed to behave elastically and then yields freely when the yield stress is reached. This is the definition of an elastic-perfectly plastic rheology. It is this part of the lithosphere which transmits the high stresses associated with the bending. At depths greater than 50 km temperatures are assumed to be sufficiently high that thermally-activated creep processes (that is, dislocation creep) relieve the elastic stresses. Predicted trench topography agrees well with observations.

The primary objection to the application of the elastic-perfectly plastic rheology is the high stress level required. Many seismologists object to high stresses (greater than a few kilobars) in the lithosphere. Seismic studies indicate that the stress drops in most large earthquakes are of the order of 100 bar or less. Stress fields derived from the surface strain associated with earthquakes give similar values. On the other hand, scientists who study the mechanical behaviour of rock in the laboratory tend to favour high stresses. Mantle rocks tested in the laboratory can easily be stressed to 5 kbar without failure. Extrapolations of laboratory derived friction laws and creep studies also give high stresses in the lithosphere.

An alternative approach to the problem of the bending of the oceanic lithosphere at ocean trenches has recently been given by Melosh and Raetsky (*Geophys. J.* 60, 333; 1980). These authors postulate that the oceanic lithosphere is made up of a near-

surface elastic layer with a thickness of 15–30 km overlying a highly viscous layer 50–80 km thick. The shape of the bending lithosphere is primarily attributed to the viscous flow in the lower lithosphere. The viscous flow is attributed to solid-state creep. Again good agreement with the observations is obtained. The maximum stress required is a few hundred bars.

The principal advantage of the viscous rheology is the lower required stress level. However, the derived viscosities of 2×10^{22} – 10^{23} poise are quite low, being less than an order of magnitude larger than the viscosity associated with solid-state creep in the deeper mantle. This does not seem to be consistent with the temperature dependence of thermally activated creep processes.

It is clear that comparisons with observations at oceanic trenches cannot differentiate between the elastic-perfectly plastic and elastic-viscous hypotheses. The alternative rheologies must be applied to other flexure problems. Examples include the flexure of the lithosphere under the load of oceanic islands, the load of sediments, and the load of mountain belts. It is implicit in the viscous analysis that bending stresses will relax with time. Whether the resultant deformation of the lithosphere when subjected to a fixed load can be observed is also a subject of controversy.

An understanding of the bending behaviour of the lithosphere under load will help in our understanding of how stress is transmitted through it. An essential part of this understanding is the stress level present. At present the stress level at depth in the lithosphere is uncertain by more than an order of magnitude. Only when we understand the state of stress in the lithosphere will we be able to start to understand in some detail why earthquakes occur. □



100 years ago

BAUMGARTNER, the inventor of a navigable balloon, having three cars attached, each with ten or twelve wings, set in motion by a crank, has attempted an ascent at Leipzig. On the rope being cut the balloon rose very slowly, skimming the house-tops, whereupon the two assistants jumped out of the centre car in alarm. The balloon shot up to a great height, then burst and fell. Baumgartner was not seriously hurt, and is resolved on a second experiment.

From *Nature* 21, 8 April, 549; 1880.

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REVIEW ARTICLE

Peptidergic neurones

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More than 20 peptides have been identified in neurones of the brain, spinal cord and periphery. In several cases a peptide occurs together with a 'classical' transmitter in the same neurone. This raises new questions about the process of neurotransmission and may have important implications for our understanding and treatment of mental disorders.

It is generally accepted that nerve impulses are transferred either from one neurone to another or to an effector cell by chemical neurotransmitters released at the neuronal synapses. In 1963, McLennan¹ listed seven chemically identified putative transmitters including acetylcholine (ACh), γ -aminobutyric acid (GABA) and monoamines such as noradrenaline and dopamine. As, crudely speaking, neurotransmitters act in only two ways—to excite or to inhibit the postsynaptic cell—seven candidates seemed more than sufficient. Therefore, the recent discovery that many peptides also seem to act as transmitters in the nervous system^{2,3} has evoked both interest and doubt.

Doubts arise not only about the need for more than 20 peptide neurotransmitters, given that one excitatory and one inhibitory transmitter should be sufficient to operate the nervous system, but also because 'classical' transmitters have always been small molecules (molecular weight ~ 200), whereas some of the peptides consist of up to 30 or more amino acids (MW $\sim 3,000$). Furthermore, many peptides now identified in the brain were previously thought of as peripheral hormones, such as angiotensin II^{4,5} and members of the gastrin/cholecystokinin (CCK) family^{6,7}. Even some of the large pituitary hormones, prolactin, adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH) and growth hormone, and also the pancreatic hormones insulin and glucagon, may be present in brain (see ref. 8). But the idea of a compound being both a hormone and a neurotransmitter should not be so surprising as we have long accepted such a dual role for noradrenaline⁹.

The realisation that mammalian neurones produced and released peptides followed du Vigneaud's characterisation of oxytocin and vasopressin as octapeptides¹⁰. These two hormones are released into the blood in the posterior pituitary from the end of neurones which originate in the supraoptic and paraventricular nuclei of the hypothalamus. These neurones, previously described by the Scharfers, Bargmann and others¹¹ as neurosecretory, magnocellular neurones, were thus the first peptide neurones identified in the mammalian brain. The next peptide neurones to be discovered were those containing the three hypothalamic hormones, thyrotropin releasing hormone (TRH), luteinising hormone releasing hormone (LHRH), and growth hormone release-inhibiting hormone (somatostatin)^{12,13}. These neurones are also hypothalamic in origin, they also secrete their content into blood vessels and are therefore also neurosecretory. Neuroanatomically these systems have been assumed to correspond to the parvocellular tuberoinfundibular neurones described by Szentagothai *et al.*¹⁴.

The major impetus for the study of neurones containing either the hypothalamic peptide hormones or the host of other chemically identified peptides came when the peptides became available in amounts sufficient for production of antibodies. This made it possible to measure peptides in tissues and body fluids by radioimmunoassay¹⁵ and to localise peptides at

the cellular and ultrastructural level using immunocytochemistry.

It is important to realise that, whereas a transmitter role seems well substantiated for a few peptides, crucial experimental evidence is lacking for many others, and additional functions of neuropeptides, as trophic factors or factors involved in long-term events, should be considered. It is, however, not the aim of this review to discuss the evidence for peptides as transmitters (see refs 16, 17). Instead, we shall concentrate on the morphology and distribution of some peptide systems, as revealed by immunocytochemistry, and what this may tell us of possible functions. Of particular interest has been the discovery of peptides in neurones which also contain a classical transmitter such as noradrenaline, serotonin (5-HT), dopamine or ACh¹⁸. This is a situation similar to the one seen in certain endocrine cells, which belong to the APUD (amine precursor uptake and decarboxylation) system as defined by Pearse¹⁹ and which store both a peptide hormone and a biogenic amine, further underlining the close relationship between the endocrine and nervous systems.

The immunohistochemical technique

Immunohistochemistry²⁰ has evolved as an extremely useful technique for visualising different types of compounds in nervous (and other) tissues^{21–23}. In principle any substance can be traced provided that (1) it is available in pure form, (2) it is immunogenic (or can be rendered immunogenic by conjugation to carrier protein) and (3) it can be retained in tissue sections during processing for immunohistochemistry. In addition to this 'universal' applicability, this method has the advantage that it can be combined with other types of histochemical techniques. Furthermore, 'double-staining' techniques^{24,25} allow visualisation of more than one substance in a given section.

With the immunohistochemical approach antisera raised against peptides conjugated to carrier proteins such as bovine serum albumin, are applied to tissue sections and visualised by fluorescent or other types of markers. Various modifications can be used: the classical indirect immunofluorescence technique of Coons and collaborators²⁶; the peroxidase technique²⁷; and the sensitive peroxidase–antiperoxidase (PAP) technique²⁸. Apart from limitations of sensitivity, and the limited ability of the antibodies to penetrate to intracellular storage sites, the major problem is the specificity of the immunological reaction. Thus, an antiserum may not only react with the proper antigen but also cross-react with structurally similar peptides. Hence the cautionary use of terms such as 'substance P immunoreactive' and 'substance P-like immunoreactivity'. Future research may show that, when multiple systems are described with a particular antibody, they are not homogeneous with regard to their peptide content. Thus, for example, of the more than 30 groups of

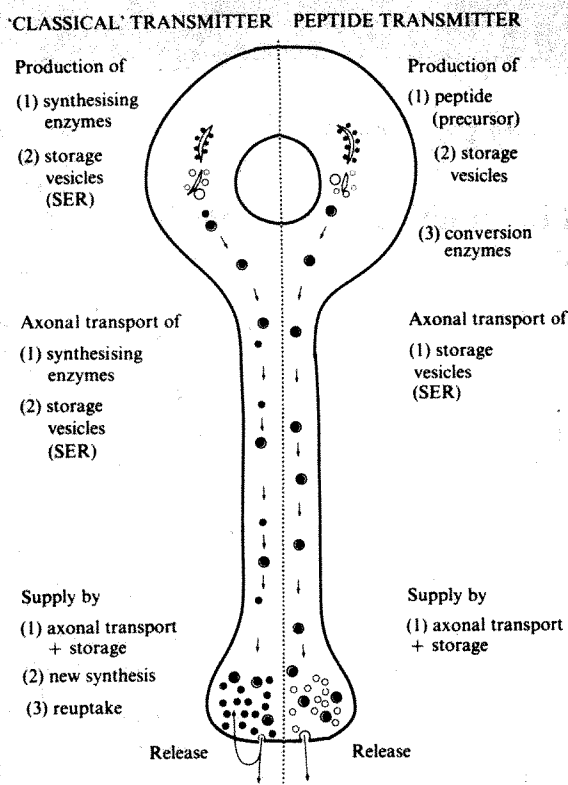


Fig. 1 Schematic drawing of a neurone demonstrating some differences between a neurone using a 'classical' transmitter such as noradrenaline (left) and a peptide transmitter (right). SER, smooth endoplasmic reticulum.

substance P immunoreactive cells found in the rat central nervous system (CNS)²⁹, perhaps only a fraction of them contain the genuine undecapeptide³⁰.

The complexity of this problem can be well illustrated by the opioid peptides³¹⁻⁴⁶. Hughes *et al.*³¹ originally discovered two pentapeptides—Leu- and Met-enkephalin—but by now several other larger opioid peptides have been shown to contain one or other of these pentapeptide sequences³¹⁻³⁶. Using antisera to Leu- and/or Met-enkephalin, many groups have described the central cellular localisation of 'enkephalin' neurones³⁷⁻⁴⁴. Because of the problem with cross-reactivity, it was not possible to decide whether the two pentapeptides were present in separate or in the same neurones, and only recently have Larsson *et al.*⁴⁴ provided evidence that Leu- and Met-enkephalin neurones constitute separate systems. It remains to be established to what extent the 'enkephalin' neurones contain the pentapeptides, as opposed to the large opioid peptides from which, in some cases, they are likely to be derived. It is clear, however, that a separate β -endorphin system exists in the rat brain^{45,46}.

A further problem with the immunocytochemical mapping of peptide neurones comes in visualising peptides in the cell soma and axon. This is probably because they contain peptides either in low concentration or in precursor form. In the latter case an antibody cross-reacting with a precursor is an asset rather than a hindrance. Thus, using one of our somatostatin antisera, cell bodies can easily be identified by means of a strong immunoreaction localised in the Golgi apparatus⁴⁷. This particular antibody is known to cross-react with a probable precursor molecule⁴⁸ and it is tempting to speculate that the Golgi-associated somatostatin-immunoreactive pool represents the precursor. Another approach for visualising peptides in cell somata is the use of colchicine, a drug which inhibits axonal flow^{49,50} and in this way increases peptide levels. Peptides in axons may be detected by ligation of nerves⁵¹ and the resulting accumulation of the peptide.

Some characteristics of peptide neurones

Only limited information is available on the structural, biochemical and physiological properties of peptide neurones. Nevertheless, it seems that they do not have unique characteristics which would allow their identification on pure morphological grounds. Peptide neurones may be small or large, multipolar or pseudo-unipolar, their nerve endings may make morphologically defined synapses or they may act on receptors over 'long' distance and they may have long or short projections. Nor does ultrastructural analysis reveal any fine structural features exclusively characteristic of peptide neurones. One feature that is common to several classes of peptide neurone is the large granular vesicle⁵², a type originally described in peripheral adrenergic neurones⁵³.

Although functional evidence suggests that at least some peptides act as neurotransmitters^{2,3,16,17,54}, the method of replenishment of peptide neurotransmitters to nerve endings seems to be different from that of classical neurotransmitters (this difference is shown schematically in Fig. 1). For example, intraneuronal noradrenaline levels are kept fairly constant by the efficient replacement of released transmitter by (1) enzymatic synthesis in the nerve endings, (2) re-uptake from the extraneuronal (synaptic) space through an active membrane mechanism and (3) supply of amine in storage vesicles (or their precursors) from the cell body via axonal transport⁵⁵. Peptides, on the other hand, are probably produced only on the ribosomes of the cell soma, possibly in the form of a larger precursor molecule⁵⁶ without local synthesis in nerve endings. As no reuptake mechanisms seem to operate for peptides in nerve endings, every single peptide molecule released from a nerve ending must be replaced by axonal transport. This comparatively inefficient and slow mechanism should be reflected in the dynamics of synaptic events at peptide synapses⁵⁷. Perhaps peptides are released intermittently rather than tonically. Such an intermittent release may, however, be 'compensated' for by a long duration of action. The amounts of peptide released may also be much smaller than those of classical transmitters. This would be in line with the rather low concentrations of peptides found in the CNS (~1,000 times less than monoamines and 100,000 times less than amino acids). Furthermore, peptides may activate receptors at much lower concentrations than the classical transmitters, a situation which may compensate for an 'inefficient' replacement of released transmitter (see below).

Distribution of peptide neurones

Early studies both with radioimmunological and immuno-histochemical techniques indicated that the three hypothalamic hormones—TRH, LHRH and somatostatin—were present outside the median eminence and even outside the hypothalamus. The finding of these neurosecretory products, previously assumed to be exclusively involved in pituitary control, also in, for example, the spinal cord was followed by reports of the presence of so called gut hormones such as vasoactive intestinal polypeptide (VIP)⁵⁸⁻⁶⁰ and CCK⁶ in many areas of the brain. A hypothesis has since emerged according to which the central and peripheral nervous systems and the endocrine system have a number of peptides, or perhaps families of peptides, in common⁶¹. Although differences can be observed between various species, the occurrence of peptide-containing neurones is widespread.

Central peptide neurones

More or less extensive maps of the many central peptide systems have been published: for immunoreactive substance P^{29,62}, enkephalin³⁷⁻⁴⁴, neurotensin⁶³, CCK⁶⁴⁻⁶⁶ and bradykinin⁶⁷. Although they overlap in many areas, each central peptide system has a unique distribution pattern of cell bodies. For example, although both substance P and enkephalin immunoreactive cell bodies have been described in more than 30 areas of the CNS, they overlap only slightly and, when present in the same nuclei, the cells do not seem to be identical.

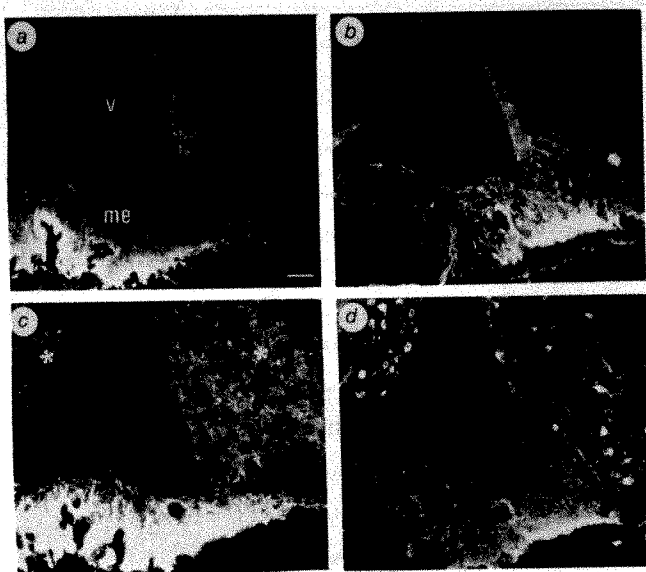


Fig. 2 Immunofluorescence micrographs of four serial sections incubated with antiserum to TRH (a), LHRH (b), somatostatin (c) and tyrosine hydroxylase, a marker for catecholamine neurones (d). All four substances are present in the median eminence (me) close to portal vessels (arrows), but with clearly different distribution patterns indicating their occurrence in separate neurone systems. Note dense plexus of somatostatin in nerve fibres also outside the median eminence in the arcuate nucleus (asterisks) suggesting a transmitter role. Of these four systems only the dopamine neurones have their cell bodies in the basal hypothalamus (d). V, ventricular. Scale bar, 50 μ m (from ref. 181).

Coexistence of two peptides has, however, been described. Immunoreactive ACTH and β -endorphin are present in the same neurones in the hypothalamus⁶⁸⁻⁷⁰, and recently we have observed TRH-like immunoreactivity in substance P (and 5-HT) immunoreactive neurones of the medulla oblongata^{18,71}.

It is clear from the data collected so far, that some areas of the brain are rich and others are poor in peptide-containing neurones. The hypothalamus, the amygdaloid complex (particularly its central nucleus), certain nuclei of the medulla oblongata and the dorsal horn of the spinal cord are examples of areas of the rat brain where most peptides can be found. In the cerebellum, on the other hand, only scattered peptide fibres are observed. The cerebral cortex seems to lack many peptides but is rich in somatostatin-, VIP- and a gastrin/CCK-like peptides, all present in interneurones⁷². Of particular interest are the median eminence of the hypothalamus and the dorsal horn of the spinal cord.

The first immunohistochemical identification of a small peptide in neurones was of LHRH-immunoreactive fibres observed in the external layer of the median eminence of the hypothalamus⁷³⁻⁷⁵. TRH and somatostatin fibres were also later found in this brain region (Fig. 2). Lesion studies suggest that these three peptides do not originate in the basal hypothalamus, as assumed originally, but deeper within the hypothalamus⁷⁶. Instead, the arcuate neurones with short projections to the median eminence seem to contain small classical transmitters such as dopamine⁷⁷, GABA⁷⁸ or ACh⁷⁹, which may exert part of their action at the median eminence level rather than being released into portal vessels. It may be, therefore, that a set of short tuberoinfundibular dopamine, GABA and ACh neurones act as a type of 'gating system', controlling the release of hypothalamic hormones into the portal vessels via an axo-axonic influence⁷⁶.

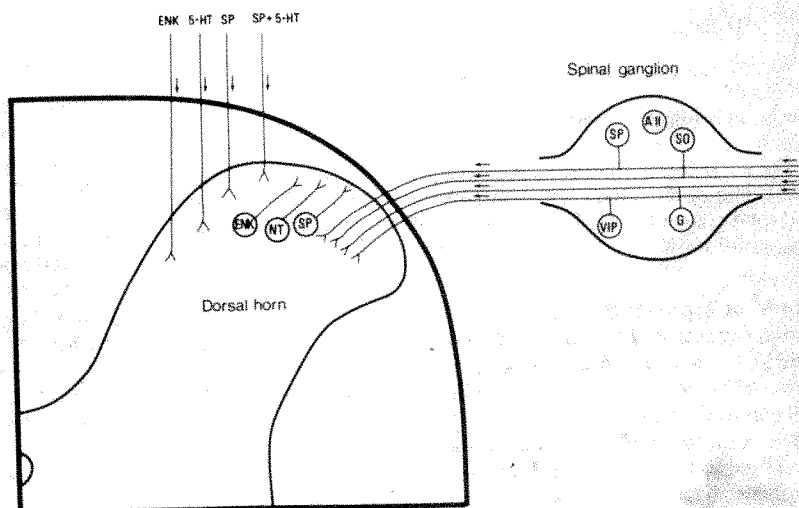
The dorsal horn of the spinal cord receives a complex input of peptide-containing neurones as seen in Fig. 3. Immunoreactive substance P, somatostatin, VIP and gastrin/CCK are present in primary sensory neurones^{65,80,81}. Substance P (and perhaps somatostatin) is also observed in propriospinal neurones or spinal interneurones^{29,82}. Whether the supraspinal substance P/5-HT neurones, which descend to the ventral horn⁸³, also project to the dorsal horn is not yet firmly established. A descending enkephalin-immunoreactive system⁸⁴ may also project to the dorsal horn. Neurotensin⁶⁴ and enkephalin^{38,85} are present in interneurones (or possibly propriospinal neurones). These findings suggest that peptides play an important part in the processing of sensory information at the spinal level.

Peripheral and sensory peptide neurones

The wide distribution of peptides in peripheral neurones has now been firmly established. Initial observations concerned substance P in primary sensory neurones⁸⁶⁻⁸⁸, but this and other peptides are also present in virtually all parts of the autonomic nervous system, particularly the gut and certain autonomic ganglia. There are well documented examples of primary sensory neurones containing immunoreactive substance P, somatostatin⁸⁹, VIP⁸¹ and gastrin/CCK^{65,81}. It is not yet absolutely clear whether these peptides can ever occur in the same neurones, but substance P and somatostatin at least do not⁸⁹. Substance P-like immunoreactivity has also been observed in the vagus nerve^{81,90-94} and in taste neurones of the cat⁹⁵. Thus, substance P may be involved in sensory processes of various types, including pain⁹⁶.

If substance P is a transmitter substance in primary sensory neurones one might expect its main action to be exerted at the central branches in the spinal cord or brain stem. A role for

Fig. 3 Schematic illustration of a spinal ganglion and the dorsal horn of the spinal cord and some of its peptide systems. Note that substance P (SP) probably is present in three, possibly four different systems: (1) primary sensory neurones; (2) interneurones or propriospinal neurones; (3) descending systems, some of which may contain both SP and 5-HT. AII, angiotensin II; ENK, enkephalin; G, gastrin/CCK; NT, neurotensin; SO, somatostatin; VIP, vasoactive intestinal polypeptide. In the dorsal horn there are other peptide and non-peptide systems, not mentioned here (from ref. 80).



substance P in the periphery has, however, long been suspected, for example in the axon reflex inducing antidromic vasodilation⁹⁶⁻⁹⁸. Recently, Olgarth *et al.*⁹⁹ have demonstrated that substance P can be released from peripheral, presumably sensory, branches in the tooth pulp. Interestingly, more than 90% of the substance P immunoreactive material produced in the nodose ganglion (of the vagus system) seems to be transported into the peripheral branch⁹⁴. All these findings suggest that substance P may have an important function at its sensory terminal branches by, in fact, acting at 'postsynaptic' cells or perhaps by modulating the threshold of 'its own' nerve ending. The occurrence of substance P in presumptive taste neurones also indicates other possibilities. It is well known that the structural integrity of the taste buds is dependent on their sensory innervation¹⁰⁰. Transplantation experiments have shown that different types of sensory ganglia, even lumbar sensory ganglia, can induce taste bud formation^{101,102}. In all these ganglia substance P is present. Could it be that this peptide is of trophic importance for the structural integrity of the taste buds?

Autonomic ganglia: Autonomic ganglia, particularly prevertebral sympathetic ganglia in the guinea-pig, contain networks of peptide immunoreactive fibres (Fig. 4). Thus, the inferior mesenteric ganglion has dense plexuses of enkephalin¹⁰³ and VIP¹⁰⁴ immunoreactive fibres and in addition substance P¹⁰⁵, gastrin/CCK and bombesin (ref. 65 and our studies, in preparation) positive fibres are present. A few somatostatin-immunoreactive fibres are also seen¹⁰⁶. Ligation and transection experiments have revealed that some, at least, of these peptides are present in separate systems (ref. 80 and Lundberg *et al.*, in preparation). Thus the enkephalin-immunoreactive fibres

Table 1 Examples of neurones and endocrine cells containing both a classical transmitter and a peptide

Classical transmitter	Peptide	Area (species)	Refs
Serotonin	Substance P	Medulla oblongata (rat)	83, 134-137
Serotonin	TRH	Medulla oblongata (rat)	18, 71
Serotonin	Substance P + TRH	Medulla oblongata (rat)	18, 71
Noradrenaline	Somatostatin	Sympathetic ganglia (guinea pig)	106
Noradrenaline	Enkephalin	SIF-cells (cat)	138
		Sup. cerv. ganglion (rat)	103
		Adrenal medulla (many species)	138, 139
Noradrenaline	Neurotensin	SIF-cells (guinea pig, cat)	103, 138
		Adrenal medulla (cat)	138
Noradrenaline	Somatostatin	Adrenal medulla (man)	140
Adrenaline	Enkephalin	Adrenal medulla (many species)	138-140
Dopamine	Enkephalin	Carotid body (cat, dog, monkey)	141
Dopamine	CCK	Ventral tegmental area (A9, A10) (rat, human)	18, 142
Acetylcholine	VIP	Autonomic ganglia (exocrine glands) (cat)	143, 144

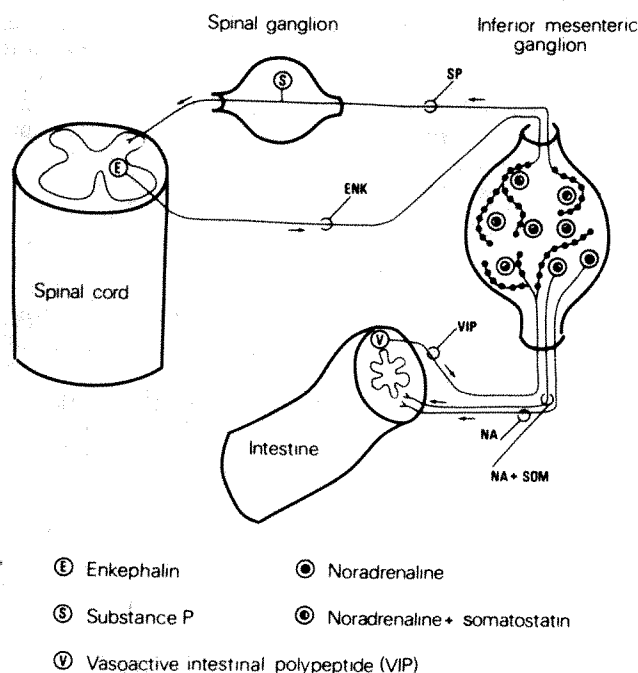


Fig. 4 Schematic illustration of some peptide systems connecting the inferior mesenteric ganglion with other parts of the nervous systems (guinea pig). There may in addition be a gastrin/CCK projection from the gut to the ganglion (from ref. 80).

represent preganglionic neurones, the substance P-immunoreactive ones are of a sensory nature, the VIP and gastrin/CCK fibres probably originate in the gastro-intestinal tract, whereas the somatostatin fibres have their origin within the ganglion itself. Some peptide projections could very well correspond to neurones first described by Kuntz¹⁰⁷ and recently investigated by Kreulen and Szurszewski¹⁰⁸ and assumed to be involved in reflexes between enteric mechanoreceptors and

sympathetic prevertebral neurones. Of particular interest are the presumably sensory fibres containing substance P, which may represent collaterals from axons on their way to the gut. A similar arrangement has been proposed for the nervous supply of the nasal mucosa¹⁰⁹. If substance P is released from such collaterals, this could explain the need for a considerable transport of substance P into the peripheral branches of the sensory neurones (see above). In this context it is of interest that Krier¹¹⁰ has demonstrated that substance P increases the excitability of nerve cells in certain sympathetic ganglia.

Gastro-intestinal tract: Peptide neurones containing immunoreactive substance P¹¹¹⁻¹¹⁴, VIP¹¹⁴⁻¹¹⁶, somatostatin^{114,117,118}, enkephalin^{37,114,119,120}, neurotensin¹¹⁴, gastrin/CCK^{65,114} and bombesin¹²¹ have been identified in the gastro-intestinal tract. Analysis of neurones stained consecutively with different antibodies has revealed that substance P, VIP and enkephalin are present in separate systems¹¹⁴. In the proximal colon the somatostatin- and gastrin/CCK-like peptides coexist in some neurones, although separate somatostatin and gastrin/CCK neurones seem to exist¹¹⁴. Most of these peptide systems are intrinsic to the gut¹¹⁴, but morphological and functional analysis is complicated by the fact that the same peptides are also present in extrinsic neurones of, for example, the vagus nerve.

Tracing peptide pathways

Although the localisation of peptide-containing cell bodies and nerve terminals is now well documented, comparatively little is known about the connecting axons because their peptide content is usually too low to detect. Similar problems with catecholamine axons were solved by transection experiments in which the pile up of transmitter on the cell body side of the lesion allowed mapping of the axon pathways¹²². Even so, a very large number of consecutive sections must be processed in order to follow long pathways, for example, from the lower brain stem to the forebrain. Anterograde and retrograde tracing techniques¹²³ helped overcome this limitation. In particular, retrograde peroxidase tracing^{124,125} combined with immunochemical 'staining' with antiserum to tyrosine hydroxylase¹²⁶ has enabled the projection of dopaminergic neurones to be mapped. More recently we have combined retrograde fluorescent dye tracing¹²⁷ with peptide immunohistochemistry¹²⁸ to map, for example, an

enkephalin-immunoreactive pathway from the lower medulla oblongata to the spinal cord⁸⁴.

Coexistence of peptides and 'classical' transmitters

The coexistence of peptides and amines is well known in endocrine cells, particularly in the gastro-intestinal tract^{19,129-131}. According to Pearse, these cells belong to the APUD system, the cells of which are "neuroendocrine-programmed cells originating from the ectoblast"^{19,132,133}. As neurones have the same embryonic origin it was postulated that they also may contain both a peptide and an amine. The fact that several examples have been observed in a relatively short time (Table 1) may indicate the generality of this situation^{18,71,83,103,106,134-144}. Further examples may be found when new techniques or new markers for other types of neurones have been developed. If so the apparent wealth of 'new' peripheral (and central) peptide neurones may, in fact, only reflect 'old' systems, which in addition to their classical transmitter contain a peptide. Perhaps it will turn out that there are no neurones that contain 'only' peptides.

The possible occurrence of two transmitters in the same nerve terminals raises the question of their subcellular storage sites (Fig. 5). In most types of nerve endings, small (diameter about 500 Å) and large (diameter about 1,000 Å) granular vesicles can be distinguished. Ultrastructural immunocytochemical studies indicate that peptides are usually present in the large vesicles⁵². No ultrastructural immunocytochemical studies have been performed on nerve endings identified and characterised as containing both a peptide and an amine (or ACh). It may, however, be speculated that, as monoamines seem to be present both in small and large vesicles^{145,146}, peptides and amines may coexist in the large vesicles, whereas the small ones may contain only the amine¹⁴⁷. A differential subcellular storage compartment is, however, suggested by the fact that even very high doses of reserpine, which almost completely deplete the monoamine stores, do not seem to affect substance P levels^{83,148}.

It has generally been assumed that one neurone produces and releases only one transmitter, a concept often referred to as Dale's principle^{149,150}. It seems, therefore, important to understand the possible functional significance of two putative transmitters in one neurone. Note that coexistence does not follow a simple pattern. For example, substance P and gastrin/CCK is present only in a small proportion of the 5-HT and dopamine neurones, respectively, originally described by Dahlström and Fuxe¹⁵¹. Similarly, we have observed many more substance P-immunoreactive cell groups (about 30)²⁹ than those containing both substance P and 5-HT. In no case do all of the neurones that contain a particular classical neurotransmitter also contain the same peptide and vice versa. Several explanations seem possible, but an attractive hypothesis is that each type of neurone, defined by the classical neurotransmitter that it

contains, is subdivided into groups characterised by the peptide they contain. We and many others have, in the analysis of the monoamine neurones, previously been struck by their apparent widespread distribution and homogeneity. But perhaps there is really a heterogeneity in which the specific peptides or each subgroup confers the ability to convey differentiated messages.

The best neurones so far available for a functional analysis seem to be those that contain VIP-like immunoreactivity, whilst being rich in acetylcholinesterase (AChE)^{143,144}. Such neurones were first observed in lumbosacral, sympathetic ganglia¹⁴³, which contain one of the best defined cholinergic (AChE-rich and choline acetylase-containing) neurone populations and are assumed to be involved in the regulation of sweat secretion¹⁵²⁻¹⁵⁴. More recent evidence, however, suggests that VIP in cholinergic neurones may be a general characteristic of secretomotor neurones¹⁴⁴. In Fig. 6 a hypothesis for the physiological significance of such neurones is presented. It is generally accepted that ACh produces secretion by an action on the secretory cells (directly and/or indirectly via myoepithelial cells) and that this effect is atropine-sensitive^{152,155}. The concomitant vasodilation, which occurs on stimulation of the postganglionic nerves, on the other hand, is atropine-resistant¹⁵⁵⁻¹⁵⁷. On the basis of studies on colon Fahrenkrug *et al.*¹⁵⁸ have suggested that VIP may be the mediator of atropine-resistant vasodilation. It is now postulated that these two effects—the secretion and vasodilation in exocrine glands—are induced by ACh and VIP, respectively, released from the same nerve terminals to act synergistically^{143,144}. In this particular situation the two putative transmitters would thus act on receptors located on two separate cell types. Clearly, in other systems different mechanisms may operate. There is, for example, evidence that substance P may block cholinergic receptors¹⁵⁹⁻¹⁶¹. In addition, preliminary studies indicate an interaction between 5-HT and substance P at the same receptor in the spinal cord¹⁶², which would agree with the existence of nerve terminals in the spinal cord containing both these compounds⁸³.

Just as the APUD concept has been of importance for our understanding of pathological processes, for example endocrine tumours (so called apudomas) in the gut¹⁶³, so may the coexistence of transmitters be to our understanding of neurological diseases and their treatment. Of particular interest in this respect is the occurrence of a CCK-like peptide in a subpopulation of dopamine neurones in rat and man^{18,142}. In the rat these cells are located mainly in the most medial parts of the zona compacta of the substantia nigra (A9 group)¹⁵¹ and in the ventral tegmental area (A10 group)¹⁵¹ and are known to project mainly to limbic areas in the forebrain¹⁶⁴. In agreement, CCK-immunoreactive (and dopamine-containing) nerve endings are found, for example, in the nucleus accumbens and the tuberculum olfactorium¹⁴².

The occurrence of a peptide in a population of dopamine neurones is interesting in view of our extensive knowledge of the functional role of these dopamine systems. In particular, the mesolimbic dopamine systems have been associated with higher mental functions and, according to the so called dopamine hypothesis, disturbances in this system may represent one component in the pathogenesis of schizophrenia¹⁶⁵. If a CCK-like peptide is released together with dopamine, the peptide could also be involved in the aetiology and symptomatology of schizophrenia. It has recently been demonstrated that CCK fragments can inhibit dopamine release in the nucleus accumbens-tuberculum olfactorium region¹⁶⁶. Thus, the peptide released together with the 'main' transmitter dopamine may be part of an inhibitory feedback system modulating dopamine release via action on autoreceptors. We have speculated¹⁶⁶ that an imbalance between peptide and amine, in fact, could be an aetiological factor for schizophrenia, whereby a loss or decrease in peptide would lead to an 'overactive' dopamine system¹⁶⁵.

Finally, the coexistence of putative peptides and biogenic amine transmitters raises questions about substitution therapy. The belief that some mental and neurological disorders are due to degeneration of transmitter-characterised systems has

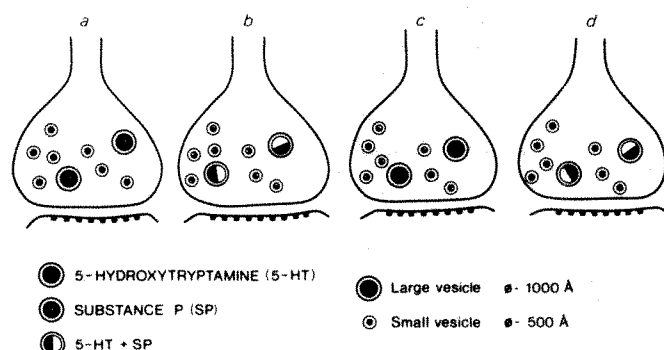


Fig. 5 Schematic illustration of four alternatives for possible storage sites of an amine (5-HT) and a peptide (substance P) coexisting in a nerve ending (From ref. 80).

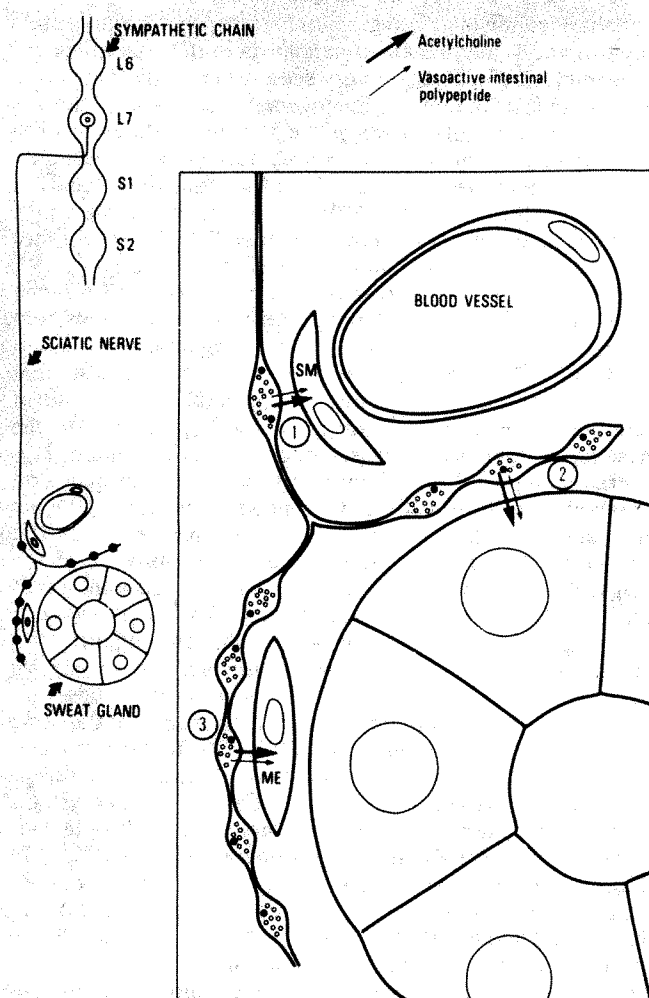


Fig. 6 Schematic illustration of our working hypothesis on the sympathetic innervation of sweat glands. Acetylcholine (thick arrow) and VIP (thin arrow) are released from the same nerve endings. Whereas VIP mainly causes vasodilation by relaxing smooth muscle cells (SM) around blood vessels (1), ACh mainly causes secretion by direct stimulation of secretory cells (2) or indirectly through myoepithelial cells (ME) (3) (from ref. 143).

formed the basis of therapy by administration of the lost transmitter (or its precursor). The best example is Parkinson's disease which is now, due to the pioneering work of Carlsson, Cotzias and Hornykiewicz and others¹⁶⁷, known to be due to degeneration of dopamine systems in the basal ganglia and which can be treated by peripheral administration of L-dopa, the precursor of dopamine. It is obvious that if a neurone releases two transmitters, substitution should, at least in certain conditions, consequently occur with both substances. It may therefore be that future substitution therapy will have to consider administration of two or more components. This argument is, however, not valid if the peptide acts on a presynaptic receptor, as discussed above for the limbic dopamine/CCK neurones. For this particular system other aspects must be considered in relation to drug therapy. Schizophrenia is routinely treated with neuroleptics assumed to exert their beneficial effects via blockade of dopamine receptors¹⁶⁸. It may be that 'dopamine overactivity' can be reduced also by administration of CCK fragments or drugs acting on the hypothetical CCK receptors on dopamine nerve endings¹⁶⁶.

Conclusion

Although our understanding of the functional role of peptides is still incomplete, interesting discoveries have been made. For example, the posterior pituitary hormones seem to be involved in memory processes¹⁶⁹, central endorphins are important in

pain mechanisms¹⁷⁰, substance P in primary sensory neurones may be involved in processing of pain and in axon reflexes causing vasodilation in skin⁹⁶, CCK octapeptide causes satiety¹⁷¹, angiotension II injected intracerebrally stimulates drinking and increases blood pressure^{172,173} and LHRH increases sexual behaviour¹⁷⁴. The effects mentioned above represent a narrow selection of the work going on in the field of neuropeptide physiology but illustrate the wide variety of functions in which peptides may be involved. In parallel work our knowledge of the electrophysiological characteristics of peptide neurones is rapidly advancing¹⁷⁵⁻¹⁷⁷.

Finally, peptides have been identified in the brain, spinal cord and periphery. Several of these peptides were already known as peripheral hormones. Thus, one and the same peptide, can be present in intestinal endocrine cells, gut neurones, sensory neurones of various types and in a large number of central systems. This may be a way of 'economising' on genetic material. At present the identification of the intraneuronal peptides rests mainly on immunological techniques. But new techniques mean that it will soon be possible to chemically characterise very small samples of peptides. The 'peptide maps' so far published may then have to be revised. Sometimes, or maybe even always, these peptides are found in neurones which also contain one of the classical transmitters, such as a catecholamine, 5-HT or ACh.

There is experimental evidence that at least some of these peptides, for example substance P, act as neurotransmitters. However, comparatively little is known about their exact physiological role. This is partly due to a lack of drugs which can influence peptide induced events at synapses. The wealth of such drugs discovered and developed for studies of monoaminergic mechanisms has been an important reason for the rapid advancement of our understanding of the roles of dopamine, noradrenaline and 5-HT neurones in the brain in normal and pathological conditions^{178,179}. Whilst awaiting the development of equivalent drugs for peptides, some results can be expected from the use of antisera which block peptide-induced effects^{144,180}.

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ARTICLES

Diffusion of light hydrocarbons through near-surface rocks

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The sharply decreasing concentrations for individual light hydrocarbons over the near-surface intervals from several organic matter-bearing shale outcrops indicate that diffusion towards the lithosphere/atmosphere interface is a continuous process. Diffusion coefficients calculated are between 10^{-6} and 10^{-8} $\text{cm}^2 \text{s}^{-1}$ for propane to heptane-range hydrocarbons.

ORGANIC matter in sedimentary rocks which occurs primarily in a solid finely disseminated condition¹ is often very inhomogeneously distributed by interbedded rich and lean strata. Molecular diffusion of light hydrocarbons, generated by burial and temperature increase, through water saturated rock pore spaces should, therefore, be widespread in those sedimentary sequences. Diffusion of propane to heptane-range hydrocarbons through the caprock of a reservoir oil accumulation has been observed². However, except for experimental measurements³ no diffusion rate data are available for light hydrocarbons through rocks in natural conditions; this is partially due to inadequate subsurface sampling. Diffusion of light hydrocarbons is also most likely to occur at the exposed surface of an organic matter-rich shale which, before uplift, had been buried deep enough for hydrocarbon generation. Diffusion processes should be active within the near-surface interval along concentration gradients towards the interface between lithosphere and atmosphere.

Evidence is presented here that light hydrocarbons continuously migrate by molecular diffusion through near-surface intervals of source bed-type shales and ultimately escape into the atmosphere. A mathematical model is then used to calculate diffusion coefficients for individual normal and branched alkanes in the molecular range C_2 – C_7 . This, to our knowledge, is the first case where absolute rates of diffusion of these light hydrocarbons through rocks were determined in natural conditions. The geochemical significance of these diffusion coefficients is shown by the amount of petrogenic hydrocarbons which return into the global carbon cycle by continuous slow degassing of the lithosphere.

Samples and methods

For this study 38 samples were taken at close intervals from cores of three shallow boreholes (6.70, 8.10 and 10.0 m deep) drilled vertically into outcrops of source bed-type shales. Each core hole was spudded in fresh outcrop, that is any soil, unconsolidated overburden and weathering debris were removed. Core holes B and E were drilled into outcrops of dark grey, silty shales of Upper Cretaceous Campanian/Maastrichtian-age at Itivdle and Niaqorssuaq on Nûgssuaq Peninsula, West Greenland. Core hole H penetrated dark grey, calcareous shales of Lower Jurassic Pliensbachian age at an abandoned quarry near Jöllenbeck, North-west Germany. Well consolidated shales of low porosity and permeability which showed no visible traces of weathering alterations of the minerals were found throughout the total length of each core except for a thin, nearly horizontal fracture at 7.3 m depth location H. For a 5-cm thick interval on either side of this fracture the walls of which were stained by iron oxides, the shale showed a pronounced colour change to light grey. A sample was analysed both from this zone and the unweathered dark shale 10 cm below. An outcrop sample from 0.3 m depth was also obtained at each drill site by normal digging. All samples were sealed immediately in

gas-tight tin cans on core retrieval and were stored frozen in the laboratory.

Low molecular weight hydrocarbons were analysed by hydrogen stripping combined with capillary gas chromatography⁴. A glass tube containing a freshly crushed rock sample is placed between the capillary column and the carrier gas supply. Then the sample is stripped by a measured volume of the carrier gas (hydrogen) at ambient temperature. After intermediate trapping the volatile compounds are transported by the carrier gas into the chromatograph by splitless introduction. Detailed compositional information including identification of ~32 individual hydrocarbons in the molecular range C_2 – C_8 is obtained from a 1-g sample. This new method is very sensitive, for example, the lowest detectable quantity for *n*-butane is $10^{-9}\%$ w/w sediment. The kerogen quality was analysed by a pyrolysis technique⁵.

All three shale units contain significant concentrations of organic carbon (2.7, 6.1 and 1.0% mean for core holes B, E and H respectively) and exhibit a narrow range of variation around the mean. Similarly, the kerogen in samples of all three sites is uniformly deficient in hydrogen indicating a type III quality⁶. Mean vitrinite reflectance values of 1.30, 0.98 and 1.74% for sites B, E and H, respectively, indicate that these shale units had adequate subsurface temperatures for hydrocarbon generation. The main difference between these core holes concerns the physical state of the pore water. For sites B and E, West Greenland, the presence of permafrost and thus ice filling the rock pore space can be inferred from their high latitude geographic location.

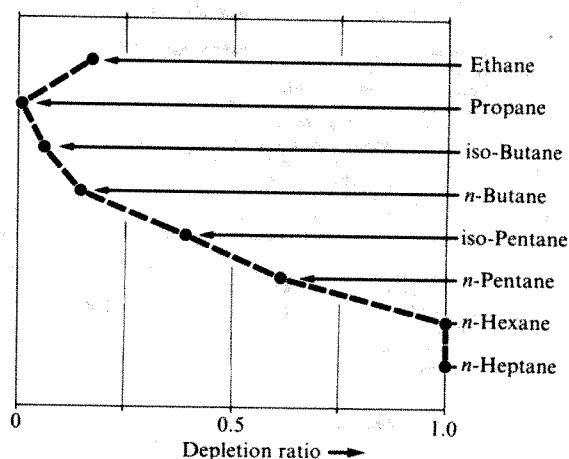


Fig. 1 Depletion ratio for selected light alkanes across near-surface interval of Campanian/Maastrichtian shale outcrop at core hole E, Niaqorssuaq, Nûgssuaq Peninsula, West Greenland. Depletion ratio is defined as the ratio of the sample concentration at 0.3 m to the mean concentration of all samples below 3 m depth.

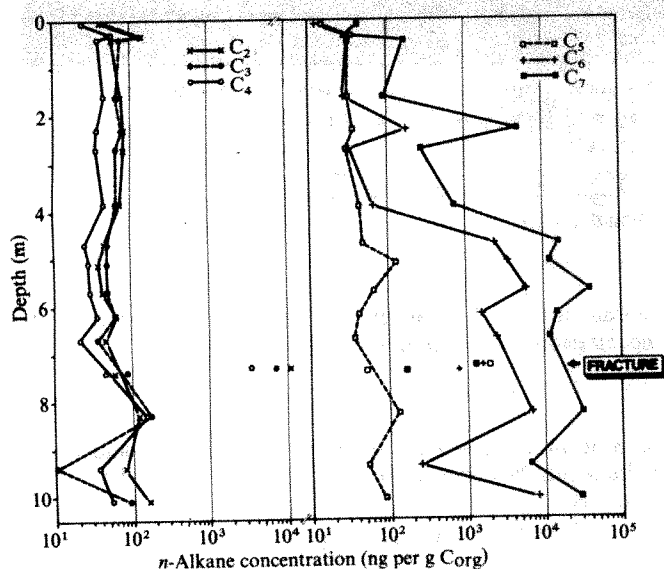


Fig. 2 Depth-plots of *n*-alkane concentrations (ng per g C_{org}) for core hole H, Pliensbachian shale, Jöllenbeck, North-west Germany. Note anomalous values for two samples close to major fracture at 7.3 m depth.

Distribution of light hydrocarbons in near-surface strata

Characteristic depth trends of individual light hydrocarbon (C_2 – C_8) concentrations in the Campanian/Maastrichtian age shales from West Greenland (core holes B and E) suggested that upward diffusion of ethane to pentane-range hydrocarbons was an active process within the near-surface 3-m interval. In particular, regular trends of sharply decreasing concentrations approaching the outcrop surface were recognised indicating a near-surface depletion zone. Details have been published elsewhere⁷. Figure 1 summarises the principal conclusions using core hole E as an example. Below a depth of 3 m a nearly constant concentration was observed for most hydrocarbons. The mean of all samples analysed from below that depth was considered to be the original generated concentration for this shale and is, therefore, assigned a value of unity in Fig. 1. To illustrate the degree of depletion of the sample closest to the outcrop surface, the concentration ratio 'outcrop sample/mean subsurface samples' is termed 'depletion ratio' and shown on the horizontal axis of Fig. 1. The outcrop sample of this shale is most drastically depleted in propane amounting to a 99.8% loss of the original concentration. The diffusive loss decreases drastically with increasing molecular size and also seems (except for ethane) to be controlled by molecular geometry. For example, the *n*-butane diffusive loss is 85.6% compared with 38.9% for *n*-pentane. Diffusion rates of each branched alkane are generally greater than that of the straight-chain isomer. Concentrations of *n*-hexane and *n*-heptane remain nearly constant with depth indicating no, or insignificant, diffusive loss.

Permafrost in West Greenland means that the physical conditions for light hydrocarbon diffusion through near-surface strata are different from those in North-west Germany, where the pore filling is liquid water. Figure 2 shows for core hole H, North-west Germany concentrations of *n*-alkanes ethane to heptane as a function of depth. With respect to the type and magnitude of the concentration changes, the molecular range can clearly be subdivided: for ethane to *n*-butane, comparatively small-scale concentration changes occur (most samples between 20 and 100 ng per g C_{org}), and seem to be independent of the distance from the outcrop surface. However, concentrations of *n*-hexane and *n*-heptane decrease sharply towards the exposure

surface and within the near-surface 5-m interval. The concentration depth pattern of *n*-pentane is between these two hydrocarbon groups. Only samples from depths of 7.3 and 7.4 m are exceptional and will be discussed separately.

For concentrations of *n*-hexane and *n*-heptane two depth intervals can be differentiated in core hole H: below a depth of ~5 m concentrations remain high and vary in a random fashion with depth, whereas in the shallow portion above they decrease continuously and by several orders of magnitude towards the outcrop surface. For example, most samples below 5 m depth have *n*-heptane concentrations between 15,000 and 30,000 ng per g C_{org} , whereas the sample from 0.3 m depth has only 20 ng per g C_{org} . As the molecular size increases this concentration decrease towards the outcrop surface covers a progressively greater range. For *n*-pentane it is <1, and it reaches ~2.5 orders of magnitude for *n*-heptane. Compared with these drastic concentration changes, those for ethane, propane and *n*-butane are minor.

This sharp decrease in concentration for *n*-pentane to *n*-heptane is interpreted to reflect an ~5-m thick near-surface interval where this shale has lost part of its original light hydrocarbon content into the atmosphere. An alternative explanation of an *in situ* origin of these depth concentration trends assuming different generation rates for these light hydrocarbons in the shallower than in the deeper portion of this core, can be dismissed. Concentration changes of this magnitude and over such short intervals could only result from a major change in hydrogen content of the kerogen^{2,7}. However, as discussed above, kerogen type is uniformly deficient in hydrogen

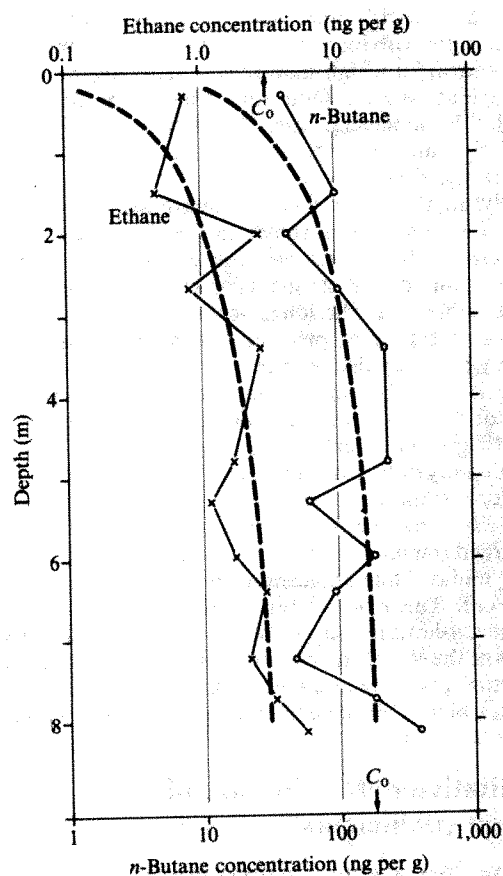


Fig. 3 Computed (broken line) and measured concentration-depth trends for selected *n*-alkanes from core hole E, Campanian/Maastrichtian shale, Niaqorssuaq, Nûgssuaq Peninsula, West Greenland. Initial concentrations C_0 used for calculation of diffusion coefficients for ethane and *n*-butane (3.1 ng per g and 165.0 ng per g respectively) are indicated by arrows.

Table 1 Absolute values of the computed diffusion coefficients ($\text{cm}^2 \text{s}^{-1}$) for selected low molecular weight alkanes in Upper Cretaceous shales from West Greenland

Core hole	Methane	Ethane	Propane	iso-Butane	<i>n</i> -Butane	iso-Pentane	<i>n</i> -Pentane	<i>n</i> -Hexane	<i>n</i> -Heptane
B	—	*	*	3.9×10^{-7}	3.5×10^{-7}	2.7×10^{-7}	1.4×10^{-7}	9.5×10^{-8}	4.2×10^{-8}
E	—	6.4×10^{-7}	1.6×10^{-6}	3.6×10^{-7}	3.2×10^{-7}	2.0×10^{-7}	1.1×10^{-7}	†	†
Recent sediments ¹⁰	3.0×10^{-6}	—	—	—	—	—	—	—	—

* Initial concentration C_0 could not be defined based on available concentration depth trend.

† Insignificant slope of concentration–depth trend towards outcrop surface.

throughout core hole H. Furthermore, a change in kerogen-type would also have to be reflected in the ethane to butane concentrations.

The rate of light hydrocarbon depletion in the shallow 5-m interval increases with molecular size producing pronounced compositional fractionation. This suggests that diffusion is the mechanism by which *n*-pentane, *n*-hexane and *n*-heptane have escaped from the near-surface 5-m interval of this shale. However, the compound separation effects in the Pliensbachian shale of core hole H are with respect to their relationship with molecular size, opposite to what would be expected from simple diffusion through porous media. The degree of depletion in the near-surface interval should be most severe for the gases and decrease with molecular size. The lack of a measurable concentration gradient for ethane, propane and *n*-butane in this shale is interpreted as an indication that only the uppermost portion of a depletion zone was penetrated, that is, the depth was not reached at which this shale has kept its original concentration for these relatively mobile hydrocarbons. The relative abundance and type of distribution of the analysed *n*-alkanes support this conclusion. A distribution envelope of continuously increasing concentrations with increasing carbon number is the opposite of what is known for a high-mature type III kerogen shale like this Pliensbachian shale². Diffusion rates seem to be strongly controlled by molecular size, that is, the shale is depleted in ethane, propane and butane to a much greater depth than for *n*-hexane and *n*-heptane.

Finally, light hydrocarbon concentrations of samples from depths of 7.3 and 7.4 m, shown in Fig. 2, deviate significantly from those of adjacent samples above and below. Both samples tend to be enriched in ethane, propane and *n*-butane, whereas they are depleted in the longer-chain *n*-alkanes. This is interpreted as reflecting the proximity of these samples to an open fracture which was visible in the core at a depth of 7.3 m. This fracture is probably in contact with the atmosphere either directly or through an interconnected fracture system. Down dip and at greater depth, ethane, propane and butane predominantly diffuse from the adjacent shale into the fracture. This hydrocarbon mixture moves upwards along the fracture, probably by a mechanism other than pure diffusion. From this gas-charged fracture ethane, propane and butane have diffused into the immediately adjacent portion of the shale causing the enrichment. The hydrocarbon mixture in the fracture lacks *n*-pentane, *n*-hexane and *n*-heptane compared with the adjacent portions of the shale. Hence those hydrocarbons diffuse towards the fracture resulting in a narrow shale interval parallel to the fracture, which is locally depleted in hexane and heptane.

Quantitative determination of diffusion coefficients

The above observations led us to calculate the absolute values of diffusion coefficients using Fick's law,

$$D \frac{\partial^2 C(z, t)}{\partial z^2} = \frac{\partial C(z, t)}{\partial t} \quad (1)$$

where $C(z, t)$ = concentration of hydrocarbons as a function of depth and time, ng hydrocarbon per g of rock; D = diffusion coefficient, $\text{cm}^2 \text{s}^{-1}$; t = time, s; z = depth, cm.

We assume that the shale unit has, at greater depth, a constant concentration, C_0 , as the initial condition

$$C = C_0 \quad \text{at} \quad \infty \geq z > 0 \quad t = 0$$

The concentration is zero at the outcrop surface, and at an infinite depth the concentration is equal to C_0 for the boundary conditions:

$$C = 0 \quad \text{at} \quad z = 0, \quad t \geq 0$$

$$C = C_0 \quad \text{at} \quad z \rightarrow \infty, \quad t \geq 0$$

Solving the diffusion equation with the above initial and boundary conditions⁸,

$$C(z, t) = C_0 \operatorname{erf} \frac{z}{2(Dt)^{1/2}} \quad (2)$$

The initial concentrations, C_0 , are determined for each *n*-alkane by calculating arithmetic or geometric means of the measured concentrations below the above defined near-surface depletion zones. Concentration values are calculated at 25-cm intervals. $D \cdot t$ is the only unknown required in the calculation of $C(z, t)$ which should correspond with the measured concentration. In mathematical terms, we try to solve an inverse problem by which one can determine the correct parameter of a system. Two techniques were used to optimise $D \cdot t$ —the least-square method, and the comparison of the areas formed between the straight lines joining the measured concentration values and the curve calculated with a certain $D \cdot t$ value. The best value of $D \cdot t$ is found with iteration that minimised the error between

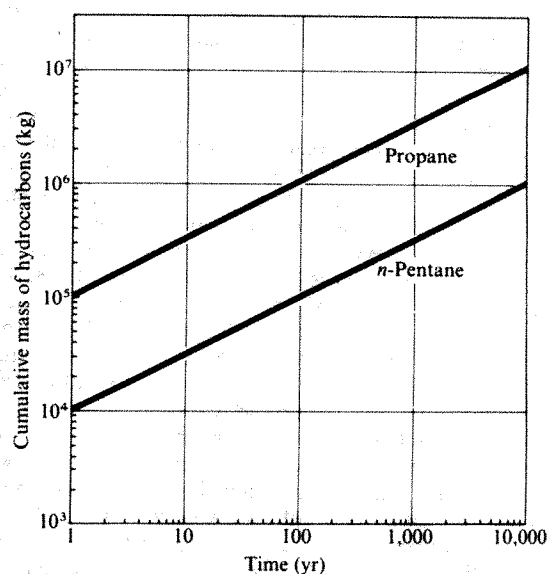


Fig. 4 Calculated cumulative mass of propane and *n*-pentane escaping as a function of time into the atmosphere from a 100 km² outcrop surface of Campanian/Maastrichtian shale on Nûgssuaq Peninsula, West Greenland.

the calculated and measured concentrations. The concentration curve found solving equation (1) is convex.

Using this model, the variation of the light hydrocarbon concentrations with depth could be calculated only for core holes B and E from West Greenland. In both cases the initial concentration C_0 can be determined with reasonable accuracy. The data of core hole H are not suitable for modelling because the diffusion front moved below the depth which was reached by the drill. Once the optimum value of $D \cdot t$ is determined, the absolute value of D is calculated by dividing $D \cdot t$ by the time available for diffusion. The initiation of diffusion for light hydrocarbons is defined here as the time when the present surface of the Earth was established at the sampling locations in the course of uplift and erosion. Although there are no direct means to measure this time exactly, the special geological and geographical conditions of West Greenland enable one to make a reasonably accurate estimate. Present topography results from isostatic uplifting and subsequent erosion following retreat of the thick ice cap which once covered coastal West Greenland. This retreat is dated at 6,000 yr BP (ref. 9), which represents a maximum value for time t to solve equation (2).

As core holes B and E were drilled into shales of the same age and the concentration-depth trends were similar, only the model results for core hole E are discussed here in detail. The concentration curves are calculated from equation (2) with optimised $D \cdot t$ values as described above and plotted together with the measured concentrations for two selected n -alkanes (Fig. 3). The similarity between the convex shape of the calculated concentration curve and the general trend of the measured concentrations strongly supports our observations that the concentration depth-trends of the n -alkanes studied is indeed mainly governed by diffusion.

It was possible to calculate $D \cdot t$ values for n - and iso-butaness and -pentanes, n -hexane and n -heptane in core hole B, and for ethane, propane, n - and iso-butaness and -pentanes in core hole E. Diffusion coefficients calculated (Table 1), vary from 10^{-6} to $10^{-8} \text{ cm}^2 \text{ s}^{-1}$. As Table 1 also shows, they compare favourably with data reported for methane although measured in recent sediments¹⁰. The calculated diffusion coefficients in core holes B and E show a strong dependence on molecular size and geometry: they decrease rapidly with increasing carbon number, except for ethane. The branched alkanes have higher diffusion coefficients than their normal homologues. Comparison of the calculated absolute diffusion coefficients for n - and iso-butaness and -pentanes in core holes B and E shows a good match (for example, for the normal alkanes 3.5×10^{-7} and $1.4 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ in B compared with 3.2×10^{-7} and $1.1 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ in E) which also indicates that the model represents a good first approximation.

Geochemical significance

Based on these absolute diffusion rates several geological processes involving hydrocarbon diffusion can now be quantified. The contribution of diffusion to primary migration of hydrocarbons, thought to involve a combination of different transport mechanisms¹¹, can now be estimated. Within the shale source beds hydrocarbons diffuse over short distances along concentration gradients towards the nearest fracture, vug or silt layer¹. Geochemical surface prospecting is based on the concept of light hydrocarbons diffusing from reservoir accumulations in the subsurface towards the Earth's surface¹². However, a reliable geochemical interpretation of such data should take into account the compositional fractionation effects documented here. Details of these geological applications will be published elsewhere.

The rate at which petrogenic hydrocarbons escape by continuous slow degassing from the lithosphere is of great interest when establishing the global carbon cycle in general and the atmospheric carbon balance in particular¹³⁻¹⁵. Based on the diffusion coefficients, the cumulative mass of propane and n -pentane escaping into the atmosphere as a function of time from

a 100-km² outcrop surface of the Campanian/Maastrichtian shale in West Greenland is calculated and shown in Fig. 4. During the initial periods, as the shale is uplifted and exposed to the surface of the Earth, high amounts of hydrocarbons enter the atmosphere per unit time, (for example, 1.1×10^5 and $1.0 \times 10^4 \text{ kg}$ in the first year for propane and n -pentane respectively), decreasing exponentially with time. However, this calculation is based on the simplified assumption that the exposure surface remains constant with time, that is that there is no continued advance of the diffusion front with progressing erosion.

Conclusions

Rocks from the near-surface intervals of three shallow core holes drilled in source bed-type shales exhibit a pronounced depletion in concentration of certain light hydrocarbons (C_2 - C_7) towards the outcrop surface. A major difference is observed in the type and depth of this depletion zone between the three cases examined. In two shale outcrops in West Greenland a depletion zone can only be recognised for ethane through pentane-range hydrocarbons and to a depth of 3 m. In North-west Germany the shale is depleted in ethane to butane-range hydrocarbons to an unknown depth which is $>10 \text{ m}$. However, for pentane to heptane-range hydrocarbons the depletion zone extends only to a depth of 5 m. This difference between West Greenland and North-west Germany reflects the influence of permafrost, that is, ice plugging the pore space retards depletion processes.

These near-surface depletion zones are explained as a result of molecular diffusion of light hydrocarbons through the water saturated pore space of the shales along concentration gradients towards the Earth surface. However, several physical factors, which have probably influenced the upward diffusion of light hydrocarbons through the shale section studied, have been neglected in the present stage of our model: adsorption of light hydrocarbons on mineral surfaces, or the inhomogeneity of the porous media, that is subtle changes with depth in clay mineralogy, porosity and permeability.

The concentration-depth trends computed by the mathematical model based on Fick's law closely match those measured for both core holes in West Greenland. An assumption of time available for diffusion in these cases makes it possible to determine absolute diffusion rates of hydrocarbons through a column of rocks in natural conditions. These diffusion coefficients vary between 10^{-6} and $10^{-8} \text{ cm}^2 \text{ s}^{-1}$ for ethane to heptane-range hydrocarbons and decrease sharply with molecular size.

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A DNA cloning system for interspecies gene transfer in antibiotic-producing *Streptomyces*

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Selectable plasmid vectors that contain a gene encoding resistance to the antibiotic methylenomycin A and that are suitable for the cloning of endonuclease-generated DNA fragments in *Streptomyces* species have been constructed and characterised.

STREPTOMYCES are Gram-positive mycelial bacteria which undergo a complex process of morphological differentiation. Because more than 60% of known antibiotics are produced by *Streptomyces* species, including many products of widespread use in human and veterinary medicine and in agriculture, these organisms hold a position of considerable medical and biological importance¹. The development of a DNA cloning system for *Streptomyces* would greatly facilitate the detailed genetic analysis of their antibiotic production pathways and of the molecular mechanisms involved in their differentiation. Furthermore, DNA cloning could produce new combinations of DNA sequences from genetically diverse organisms and perhaps result in new types and increased yields of antibiotics synthesised by these actinomycetes.

Although the process of gene exchange mediated by conjugation within actinomycetes is apparently widespread², the transfer of genetic material between individuals by such sexual means is predominantly restricted to members of the same or closely related species. The recent development of a method for protoplast fusion in *Streptomyces*^{3,4} represents a significant advance in the ability to use genetic recombination as a practical tool in the field of strain improvement; however, the production of recombinants in fused protoplasts by 'homologous recombination' is limited by the extent of DNA sequence similarity. DNA cloning methods potentially permit the insertion of a DNA sequence of any origin into a particular host strain, provided that a suitable vector system is available to propagate the cloned DNA and a means of introducing the recombinant DNA molecule into the host organism exists.

An earlier report⁵ has described a plasmid transformation system that results in the high frequency uptake of covalently closed circular (CCC) DNA by *Streptomyces* in the presence of polyethylene glycol, and allows the visual detection of transformants, after protoplast regeneration, that occur at a frequency of less than 10^{-5} . In optimal conditions, at least 20% of the regenerated protoplasts of *Streptomyces coelicolor* or *Streptomyces parvulus* were transformed with the *S. coelicolor*

plasmid SCP2* and 10^6 transformants per μg of plasmid DNA were obtained; similar results were obtained with *Streptomyces lividans* protoplasts transformed with SLP1 plasmid DNA.

The availability of an efficient system for introducing plasmid DNA into *Streptomyces* species provides a basic tool for DNA cloning in this genus. We report here the development of a *Streptomyces* cloning system using derivatives of two unrelated plasmids, SCP2* and SLP1.2.

Plasmids used for construction of cloning vectors in *Streptomyces*

Two groups of plasmids were potentially suitable for the construction of cloning vectors for *Streptomyces*:

- (1) SCP(2) and its high fertility variant, SCP2*, were first isolated from *S. coelicolor* A3(2), and both plasmids have been subjected to genetic and physical analyses⁶⁻⁸. Strains containing either of the plasmids elicit a phenomenon termed lethal zygotis when grown in contact with an SCP2⁻ culture of the same species^{6,8}. Lethal zygotis is associated with plasmid transfer and results in the retardation of the development of the SCP2⁻ culture. This phenotype proved useful in the development of a plasmid transformation system for *Streptomyces* and has facilitated the detection of low-frequency interspecific transfer of SCP2* from *S. coelicolor* to *S. lividans* and *S. parvulus*. Other *Streptomyces* plasmids have subsequently been shown to elicit and confer resistance to lethal zygotis, including SCP1 from *S. coelicolor* and the SLP1 series of plasmids from *S. lividans*. This property seems to be a fairly common phenotype among *Streptomyces* plasmids and may prove useful in identifying additional plasmids that are otherwise phenotypically cryptic. SCP2* and SCP2 have a molecular length of 31 kilobases, are present in the mycelium at about one copy per genome and have identical restriction endonuclease cleavage maps (Fig. 1) for all enzymes used.
- (2) The SLP1 series of plasmids was isolated from *S. lividans*⁹. Six different plasmid species SLP1.1-6, of molecular length

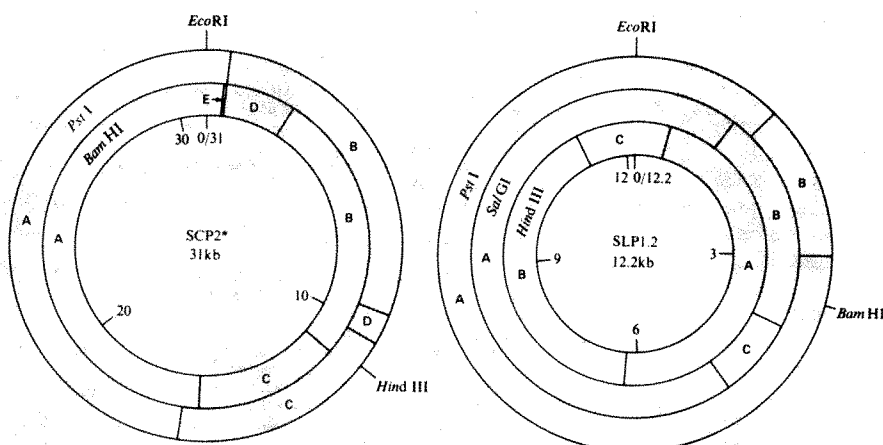
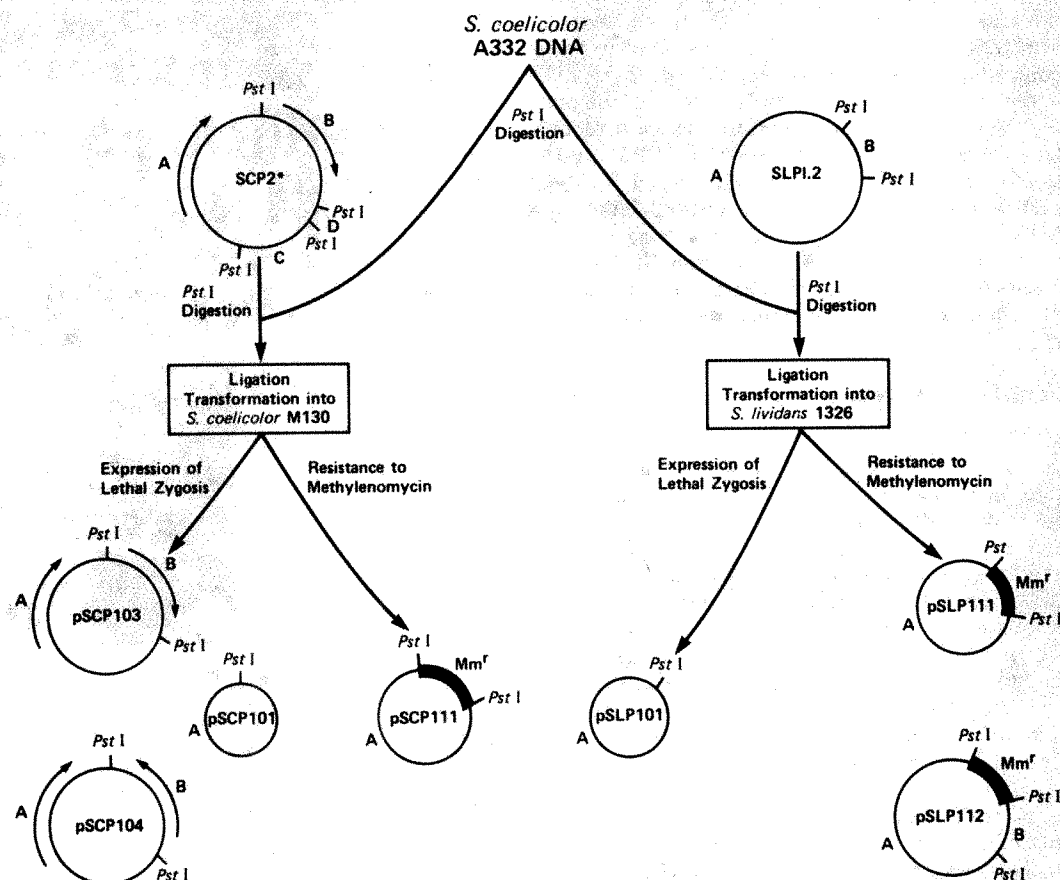


Fig. 1 Restriction endonuclease cleavage maps of the SCP2* and SLP1.2 plasmids. Distances are shown in kilobase (kb) pairs. The maps were derived from single, double and partial digests of SCP2* or SLP1.2 with the enzymes indicated. The shaded areas of each map indicate regions of the genomes which are non-essential for plasmid replication, and which include certain restriction endonuclease cleavage sites that are available for DNA insertion.

Fig. 2 Schematic outlines of the procedure used for cloning the Mm resistance gene(s) on SCP2* and SLP1.2 derived vectors. 0.5 µg of SCP2* DNA, isolated as covalently closed circular (CCC) DNA from *S. coelicolor* strain M109 and 2.5 µg of DNA isolated from *S. coelicolor* strain A332 were digested to completion with *Pst*I, mixed and ligated together at a total DNA concentration of 12 µg ml⁻¹. Similarly, 0.1 µg of SLP1.2, prepared in an identical fashion to SCP2* but from *S. lividans* M180, and 1 µg of A332 DNA were completely digested with *Pst*I, mixed and ligated together at a final concentration of 15 µg ml⁻¹. Each mixture was assayed by agarose gel electrophoresis, precipitated with ethanol, resuspended in 20 µl 10 mM Tris-HCl, pH 8, and 1 mM EDTA (TE buffer) and introduced by transformation into either 10⁷ *S. coelicolor* M130 or 2 × 10⁷ *S. lividans* 1326 protoplasts, respectively. As a control for the spontaneous occurrence of Mm^r-resistant isolates, equal numbers of protoplasts were treated with 20 µl TE buffer in an identical manner. After allowing time for protoplast regeneration and sporulation to occur⁵, spores were collected from the regeneration plates by the addition of 10 ml of sterile distilled water and gentle scraping with a wire loop, filtered through cotton wool, concentrated by centrifugation at 1,000 g for 15 min and resuspended in 0.3 ml 20% glycerol. The spore preparations were then assayed either for the presence of Mm^r clones (Fig. 3) or for expression of lethal zygosis. The remainder of the spore preparation was stored frozen at -20 °C with only slight loss of viability.



9.38–12.35 kilobases, have been isolated and shown by restriction enzyme cleavage mapping to be identical except for a single segment of the plasmid genome. As the size of the plasmid in the series increases, the length of the DNA segment comprising the variable region increases in one direction only from a fixed point. Some of the larger variants consequently acquire additional restriction endonuclease cleavage sites that are contained in DNA sequences not essential for autonomous replication. Thus, insertion of exogenous DNA fragments into these cleavage sites should not interfere with propagation of the plasmid. Particularly attractive as a potential cloning vector is SLP1.2 (12.35 kilobases), which contains a single *Bam*HI and two *Pst*I restriction sites, all within the non-essential variable region (Fig. 1). The SLP1 series of plasmids are self-transmissible and have an estimated copy number of four or five per genome (M.B., unpublished).

Only covalently closed supercoiled plasmid DNA molecules have been used in earlier transformation experiments in *Streptomyces*⁵. Preliminary experiments were therefore carried out to determine the efficiency of uptake of linear and re-ligated plasmid DNA by protoplasts in transformation conditions. SCP2* and SLP1.2 DNA were cleaved into full-length fragments with *Hind*III and *Bam*HI endonucleases, respectively, and subjected to electrophoresis on a 1% agarose gel in Tris-acetate buffer. DNA bands containing linear plasmid DNA molecules were cut out from the gel, separating these from uncleaved molecules present in the endonuclease-treated samples. Linear SCP2* DNA was purified by potassium iodide gradient centrifugation¹⁰, and the linear SLP1.2 fragment was removed from the gel slice by electroelution as described below. Treatment of linear plasmid DNA with bacteriophage T4 ligase resulted in 50% conversion to circles, as estimated by gel electrophoresis (data not shown). Ligated or unligated samples of SCP2* and SLP1.2 were then used for transformation of protoplasts of *S. coelicolor* strain M124 and *S. lividans* strain

1326, respectively, and transformants were identified by their ability to elicit lethal zygosis when placed onto a lawn of a plasmid-minus strain of the same species. Transformation frequencies in excess of 10⁵ per µg DNA were obtained for ligated DNA samples; unligated DNA gave a 10–100-fold lower transformation frequency.

Cloning of a DNA fragment determining resistance to methylenomycin A

Because neither the SCP2* nor the SLP1.2 plasmid contains a known drug-resistance gene readily usable in selection of transformants, we chose in our initial experiments to clone the gene(s) specifying resistance to the antibiotic methylenomycin A (Mm)¹¹; this would then be useful for selection purposes in subsequent cloning experiments. Methylenomycin A has antibiotic activity against a wide range of Gram-positive eubacteria and many *Streptomyces* species. Genes encoding Mm resistance as well as Mm production are present on a genetically but not physically identified plasmid, SCP1 (ref. 12). *S. coelicolor* strain A332 contains the SCP1 plasmid integrated into the chromosome and is believed to possess a single copy of the Mm resistance gene(s) per genome¹³.

The total DNA of *S. coelicolor* A332 was digested with *Pst*I endonuclease, ligated to similarly cleaved SCP2* or SLP1.2 plasmid DNA, and introduced by transformation into *S. coelicolor* or *S. lividans* protoplasts as described in Fig. 2. Spores of each strain were then tested for resistance to Mm and the ability to elicit lethal zygosis. The antibiotic produced by an SCP1-containing strain was used to select for any Mm^r clones present in a lawn of the collected transformation mixtures¹⁴.

Figure 3A illustrates the selection of Mm^r clones of *S. coelicolor*. Mm^r clones of *S. lividans* were selected in a similar way using patches of *S. lividans* SCP1⁺ strain M233 as a source of Mm. Colonies of *S. coelicolor* and *S. lividans* picked from within

the areas of inhibition of the background lawn were tested for Mm resistance and shown to be resistant to the antibiotic, in contrast to the inhibition observed for strains M130 and 1326 added, respectively, as controls to each of the plates (Fig. 3B).

When purified *S. coelicolor* Mm^r clones were replica-plated to lawns of M130 (*hisA1*, *uraA1*, *strA1*, SCP2⁻) and M110 (*hisA1*, *uraA1*, *strA1*, SCP2⁺) on supplemented R2 medium plates³, each antibiotic-resistant clone elicited lethal zygotis against M130 but not against M110, indicating the presence of an SCP2⁺-related plasmid. Similarly, the Mm^r clones of *S. lividans* also exhibited lethal zygotis characteristic of the SLP1 series of plasmids, again indicating the presence of an SLP1 derivative in these clones.

Analysis of methylenomycin-resistant plasmids

Plasmid DNA obtained from Mm^r clones of both *S. coelicolor* and *S. lividans* was isolated using caesium chloride-ethidium bromide density gradient centrifugation⁶. Gel analysis of restriction endonuclease-cleaved CCC plasmid DNA obtained from a randomly selected antibiotic-resistant clone of *S. coelicolor* indicated that it consisted of a single molecular species 18 kilobases long and contained single *Bam*HI and *Eco*RI sites. Further analysis of this plasmid species (designated pSCPIII) by endonuclease digestion and gel electrophoresis (Fig. 4) indicated that it contains the largest *Pst*I fragment of SCP2⁺ plus a novel fragment of 2.55 kilobases that presumably carries the gene(s) conferring resistance to Mm.

Similar gel analysis of the endonuclease-cleaved DNA obtained from a Mm-resistant clone of *S. lividans* revealed a plasmid species of molecular length 13.3 kilobases that also includes single cleavage sites for *Eco*RI and *Bam*HI. Additional gel analysis of this plasmid DNA species, designated pSLPIII (Fig. 4), indicated that it consists of the larger *Pst*I fragment of SLP1.2 plus a novel fragment of molecular length 2.55 kilobases. Another independently isolated Mm^r-resistant clone of *S. lividans* yielded a full-length SLP1.2-derived plasmid (pSLP112) that contained a 2.55 kilobase *Pst*I-generated fragment inserted into one of its two *Pst*I sites (Fig. 2). Hence, three independently obtained Mm^r clones all contained plasmid derivatives that included a *Pst*I-generated DNA fragment 2.55 kilobases long, suggesting that this inserted fragment included the Mm resistance gene(s). In each case the cloned *Pst*I-generated fragments lacked sites for *Bam*HI, *Eco*RI and *Hind*III.

Table 1 Stability of SCP2⁺ derivatives

Plasmid	% Loss per life cycle	
	In <i>S. coelicolor</i> strain M130	In <i>S. lividans</i> strain 1326
SCP2 ⁺	0.5 (2/420)	4 (9/224)
pSCP101	9.0 (15/168)	35 (79/224)
pSCP102	7.0 (12/168)	34 (95/280)
pSCP103	0.3 (0/336)	NT
pSCP104	0.5 (0/232)	NT
pSCP111	22.0 (50/224)	NT
	35.0 (90/254)*	NT

For all derivatives, spores were scraped from single colonies that expressed lethal zygotis when replicated onto a lawn of an SCP2⁻ strain, serially diluted and plated onto complete medium. The resulting colonies were assayed for their ability to express lethal zygotis by replication onto lawns of SCP2⁻ strains of the same species. In addition, for pSCP111, a portion of the spores from a single colony was streaked at 90° to a culture of M146 on complete medium to test for Mm resistance; this test confirmed the presence of pSCP111 in the spore that gave rise to the assayed colony. Spores from the original colony were suspended in water, serially diluted and plated onto complete medium. The resulting single colonies were then streaked against M146 to assay again for Mm resistance, which showed the continued presence of pSCP111. These plates were later replicated to lawns of M130 on supplemented R2 medium to assay for the ability of the isolates to express lethal zygotis. All Mm^r isolates expressed lethal zygotis and all Mm^s isolates did not, indicating that the loss of pSCP111 rather than DNA rearrangements resulted in a Mm-sensitive phenotype. Numbers in parentheses indicate the number of colonies not showing lethal zygotis (or for pSCP111 not showing Mm resistance) over the total number of colonies. NT, not tested.

* Loss of Mm resistance.

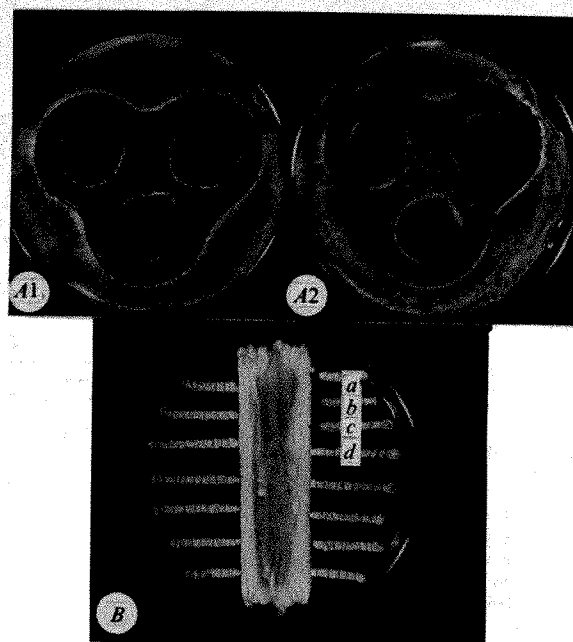


Fig. 3 Selection of Mm^r clones. A, 0.1-ml volumes of the collected *S. coelicolor* M130 transformation and control mixtures were spread onto complete medium agar²³ and the plates allowed to dry. Three patches of *S. coelicolor* SCP1⁺ strain M146 were applied to both plates, which were then incubated at 30 °C for 4–6 days: (1) transformation mixture resulting from the treatment of M130 protoplasts with TE buffer; (2) transformation mixture resulting from the treatment of M130 protoplasts with a ligated mixture of *Pst*I-cleaved SCP2⁺ and A332 total DNA. The presence of resistant colonies in the area of inhibition of the lawn produced by diffusion of Mm from the M146 patches is seen. B, Colonies growing in the zones of inhibition of plate A2 were streaked at right angles to a culture of the SCP1⁺ strain M146 on complete medium and incubated at 30 °C for 3–5 days. Aerial mycelium formation and sporulation of the Mm-sensitive control cultures M130 (SCP1⁻, SCP2⁻) (a), M110 (SCP1⁻, SCP2⁺) (b) and 1190 (SCP1⁻, SCP2⁺) (c) are inhibited proximal to the antibiotic produced by M146. Streaks of the clones picked from A2 and of strain M146 (d) were resistant to Mm.

To confirm that the novel fragment conferring Mm resistance did, in fact, contain the Mm^r gene(s) from the chromosomally integrated SCP1 plasmid, a labelled probe of pSCP111 was made by nick translation¹⁵ using [α -³²P]dCTP. This probe was then hybridised using the method of Southern¹⁶ (with modifications) to *Pst*I-cut total DNA that was isolated from two SCP1⁺ and two SCP1⁻ isolates of *S. coelicolor* (Fig. 5). The labelled pSCP111 DNA hybridised to only the *Pst*I-A fragment of SCP2⁺ (15.4 kilobases) and to DNA fragments of 2.55 kilobases present in the SCP1 containing strains A332 and M146. No hybridisation to DNA fragments of this size could be seen with the SCP1⁻ M130 and M110 DNA samples.

Interspecific expression of pSCP111

The ability to transfer genes among different *Streptomyces* species and to obtain phenotypic expression in the new host is of potential importance in the use of DNA cloning methods for the development of new antibiotics. To investigate whether the cloning system described above is likely to be of general use in *Streptomyces* species, we transformed derivatives of *S. lividans* and *S. parvulus* with pSCP111 and selected for Mm resistance using the method described previously. Protoplasts were prepared from cultures of *S. lividans* 1326 and *S. parvulus* 2283 and transformed with 10 ng of purified pSCP111 DNA. After protoplast regeneration, the resulting spore preparations were assayed for the presence of Mm^r clones using *S. lividans* strain M233 (SCP1⁺) and *S. coelicolor* strain M146 (SCP1⁺), respectively, as sources of Mm for the *S. lividans* and *S. parvulus* transformation mixtures. In both cases antibiotic-resistant colonies were obtained. Plasmid DNA was isolated from the *S. lividans* and *S. parvulus* Mm^r-resistant derivatives using caesium chloride-ethidium bromide density gradient centri-

Table 2 Fertility properties of SCP2* derivatives

Components of mating	Colony-forming units per ml on [†] :				Average recombination frequency [‡]	Plasmid transfer frequency (% per recipient)
	HUS (Parentals)	PAC	PACUS (Recombinants)	PCH		
M130 × M124	3.5 × 10 ⁹	1.2 × 10 ⁹	1.2 × 10 ²	20	3.9 × 10 ⁻⁸	—
M130 (SCP2*) × M124	5.0 × 10 ⁸	5.6 × 10 ⁸	2.1 × 10 ⁵	3.7 × 10 ⁶	3.7 × 10 ⁻³	100 (100/100)
M130 (pSCP101) × M124	6.4 × 10 ⁷	2.8 × 10 ⁸	1.4 × 10 ⁴	2.2 × 10 ⁵	1.2 × 10 ⁻³	95 (106/112)
M130 (pSCP102) × M124	1.3 × 10 ⁸	8.0 × 10 ⁷	1.2 × 10 ⁴	2.1 × 10 ⁵	1.1 × 10 ⁻³	93 (104/112)
M130 (pSCP103) × M124	1.9 × 10 ⁸	4.4 × 10 ⁸	4.2 × 10 ⁵	8.2 × 10 ⁵	2.3 × 10 ⁻³	100 (112/112)
M130 (pSCP104) × M124	2.6 × 10 ⁹	2.3 × 10 ⁹	4.2 × 10 ⁶	1.2 × 10 ⁷	3.3 × 10 ⁻³	100 (112/112)
M130 (pSCP111) × M124	3.5 × 10 ⁸	8.3 × 10 ⁸	3.5 × 10 ⁵	9.9 × 10 ⁵	1.35 × 10 ⁻³	78 (87/112)
						55 (74/134)‡

Matings were carried out as previously described²³. Plasmid transfer frequency was determined by patching colonies from the PAC plates on to minimal medium containing proline, arginine and cysteine and after sporulation replicating to a lawn of M130 spread on fully supplemented R2 medium. The colonies were then scored for their ability to elicit lethal zygosis.

§ Minimal medium containing some of the following supplements: histidine (H), uracil (U), streptomycin (S), proline (P), arginine (A) and cysteine (C).

† Expressed as the average of the mean recombination frequencies of each parent.

‡ The transfer frequency of pSCP111 was also determined by streaking colonies from the PAC plates at right angles to M146 on complete medium and scoring for Mm resistance. M130 = *S. coelicolor* hisA1, uraA1, strA1, SCP1⁺, SCP2⁺; M124 = *S. coelicolor* proA1, argA1, cysD18, SCP1⁺, SCP2⁺.

fugation and was indistinguishable from pSCP111 by gel analysis of *Pst*I endonuclease digests.

We also transferred pSCP111 from *S. coelicolor* to *S. lividans* and *S. parvulus* by mating, selecting for Mm^r exconjugants. We have demonstrated, therefore, that the pSCP111 vector can be introduced by transformation or conjugation into two different *Streptomyces* species, *S. lividans* and *S. parvulus*, and that transformants can in each instance be selected by expression of the Mm^r gene(s) cloned in the autonomously replicating pSCP111.

Plasmid derivatives of SCP2* and SLP1.2 and their properties

SCP2* is an autonomously replicating extrachromosomal element and so must contain at least one origin of replication. The plasmid is self-transmissible and presumably includes functions that accomplish its inter-bacterial transfer among different strains of *S. coelicolor*, although the precise mechanism of conjugation in these mycelial organisms is unknown. SCP2* expresses functions that determine the level of chromosomal recombination between two genetically distinguishable derivatives of *S. coelicolor* by a mechanism that is possibly related to the transfer properties of the plasmid. It also confers on its host cell production of and resistance to lethal zygosis⁶. The lethal zygosis phenotype depends on plasmid transfer, and resistance to lethal zygosis may be functionally analogous to the surface exclusion phenomenon expressed by the sex factor F in *Escherichia coli*¹⁷.

To localise the basic biological functions of the SCP2* plasmid, derivatives of SCP2* that contain only particular restriction fragments of the original plasmid were isolated. The *Pst*I-digested and ligated *S. coelicolor* transformation mixture used originally to select for pSCP111 (Fig. 2) was also diluted and plated on supplemented R2 medium to which sufficient spores of a culture of M130 had been added to produce a confluent lawn. After incubation at 30 °C for 3–4 days, growth-centred zones of inhibition of the background lawn characteristic of lethal zygosis were observed. Spores were picked from the centre of these zones of inhibition and streaked out to obtain single colonies which, on replication to lawns of M130 on supplemented R2, expressed lethal zygosis. In a similar manner, SCP2* digested with *Bam*HI endonuclease was ligated and introduced into M130, and single colonies which expressed lethal zygosis on lawns of M130 were purified.

Isolation of CCC DNA from these clones and subsequent restriction enzyme analysis using *Bam*HI and *Pst*I single and double digests confirmed unambiguously that the isolates all contained derivatives of SCP2* having the structures indicated

in Fig. 2. pSCP101 (15.4 kilobases) consisted of the *Pst*I-A fragment of SCP2*; pSCP102 (15.9 kilobases) consisted of the *Bam*HI-A fragment of SCP2* (not shown); pSCP103 (24.3 kilobases) consisted of the *Pst*I-A+B fragments of SCP2* orientated as in the original plasmid; and pSCP104 (24.3 kilobases) consisted of the *Pst*I-A+B fragments of SCP2* but in the opposite orientation.

As both pSCP101 and pSCP102 replicate autonomously, an origin of replication is necessarily located in the *Pst*I-A and *Bam*HI-A fragments of the parent plasmids. Similarly, as all these plasmids express lethal zygosis when replicated on to an SCP2⁺ lawn and also confer resistance to this phenotype when used as lawns themselves, the gene(s) involved in production of and resistance to lethal zygosis must also be located in this region.

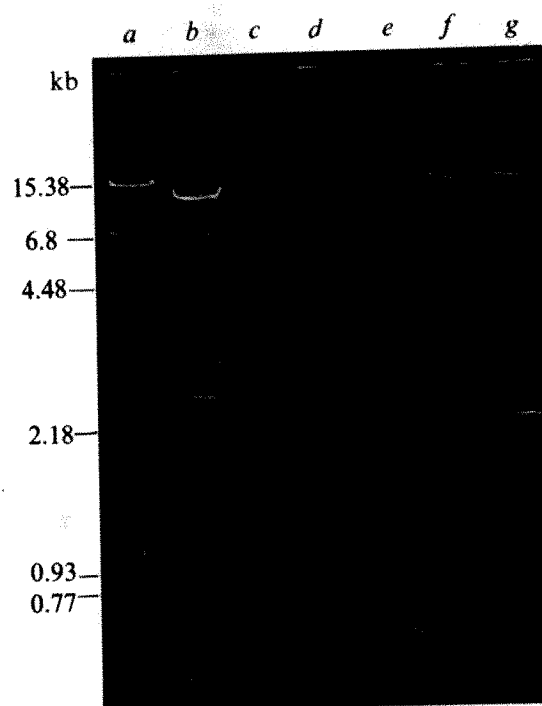


Fig. 4 Gel analysis of restriction endonuclease-cleaved pSCP111 and pSLP111 plasmid derivatives. Lanes a and g, SCP2* DNA digested with *Bam*HI and *Pst*I generating fragments of 15.4, 6.8, 4.3, 2.18, 0.93 and 0.8 kilobases. All other plasmids digested with *Pst*I. b, pSLP111; c, SLP1.2; d, pSCP111; e, pSCP101 (see below); f, SCP2*. Electrophoresis was from top to bottom in 1% agarose in 40 mM Tris-acetate, pH 7.9, and 1 mM EDTA for 4 h at 50 mA.

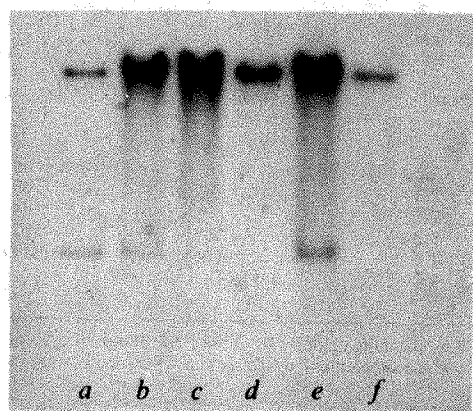


Fig. 5 Hybridisation of ^{32}P -labelled pSCP111 DNA to isolated DNA from *S. coelicolor* Mm-resistant or sensitive isolates. Aliquots (5 μg) of DNA isolated from each of the *S. coelicolor* strains A332 (chromosomally integrated SCP1, SCP2*), M146 (SCP1*, SCP2*), M110 (SCP1*, SCP2*) and M130 (SCP1*, SCP2*) were digested with 1.5 U *Pst*I at 37 °C for 3 h and electrophoresed as indicated in Fig. 4. Samples of SCP2* and pSCP111 DNA digested with *Pst*I were also included as controls. The gel was stained in 0.5 $\mu\text{g ml}^{-1}$ ethidium bromide, photographed and then soaked twice in 250 ml 0.25 M HCl for 15 min to depurinate the DNA and so enhance transfer of the larger fragments from the gel to the nitrocellulose filter²⁴. The gel was rinsed with water and soaked twice in 250 ml 0.5 M NaOH and 1 M NaCl for 15 min to denature the DNA. The gel was washed briefly three times with 200 ml water and then neutralised by soaking it for 20 min in 200 ml 3 M NaCl and 0.5 M Tris-HCl, pH 7.5. DNA was then transferred from the gel to the nitrocellulose filter soaked with $20\times\text{SSC}$, pH 6.5 ($1\times\text{SSC} = 0.3\text{ M NaCl}$, 0.03 M sodium citrate); after 5 h of contact between filter and gel, no DNA could be detected in the gel on staining with ethidium bromide. The nitrocellulose filter was baked at 80 °C in a vacuum oven for 2 h and then prehybridised at 52 °C in the presence of 50% formamide, $6\times\text{SSC}$, pH 6.5, 25 mM sodium phosphate, pH 6.5, 0.1% sodium pyrophosphate, 10 $\mu\text{g ml}^{-1}$ boiled calf thymus DNA and 1% glycine for 12 h. 0.5 μg pSCP111 DNA was incubated at 15 °C for 2.5 h in the presence of 100 μCi [α - ^{32}P]dCTP (12.5 μM), 12.5 μM each of dATP, dGTP, dTTP, 1 $\mu\text{g ml}^{-1}$ pretreated DNase and 0.1 U μl^{-1} DNA polymerase (Boehringer-Mannheim) in 50 mM Tris-HCl, pH 7.8, 5 mM MgCl_2 , 50 mg ml^{-1} bovine serum albumin and 10 mM 2-mercaptoethanol in a total volume of 20 μl . The mixture was extracted with phenol and passed over a Sephadex G-50 fine column in 20 mM Tris-HCl, pH 8, 50 mM NaCl and 1 mM EDTA to remove unincorporated label. The resulting probe had a specific activity of 1.8×10^8 c.p.m. per μg . The prehybridised nitrocellulose filter was then hybridised to 10^7 c.p.m. of the radioactively labelled pSCP111 probe, previously denatured by heating to 108 °C for 20 min in a saturated NaCl bath. Hybridisation conditions were similar to prehybridisation conditions except that $5\times\text{SSC}$ was used and calf thymus DNA and glycine were absent. Incubation was at 52 °C for 24 h in a total volume of 1.2 ml in a sealed polyethylene bag. The filter was then rinsed twice with 50 ml of hybridisation solution, incubated three times in 50 ml of the same solution at 52 °C for 1 h and washed twice for 1 h at 52 °C in hybridisation solution containing $0.5\times\text{SSC}$. The filter was finally rinsed with $2\times\text{SSC}$, dried and exposed to X-ray film for 12 h. The lanes contain: a, pSCP111; b, M146 DNA (SCP1*, SCP2*); c, M130 DNA (SCP1*, SCP2*); d, M110 DNA (SCP1*, SCP2*); e, A332 DNA (chromosomally integrated SCP1, SCP2*); f, SCP2*.

The stability of each of the SCP2* derivatives was determined in *S. coelicolor* (Table 1). The stabilities of SCP2*, pSCP101 and pSCP102 were also examined in *S. lividans* after introduction of the plasmids into this species by either mating (SCP2*) or transformation (pSCP101 and pSCP102). As shown in Table 1, the pSCP111, pSCP101 and pSCP102 derivatives were significantly more unstable than the parental SCP2* plasmid in both *S. coelicolor* and *S. lividans*. However, in *S. coelicolor*, addition of the *Pst*I-B fragment of SCP2* in either orientation to the plasmid derivative totally restored stability to the level shown by SCP2*. These observations are analogous to those made by P. A. Meacock and S.N.C. for *E. coli* plasmids¹⁸; they have isolated a region of pSCP101 that accomplishes the active partitioning of plasmid molecules in dividing cells. We suggest that the *Pst*I-B fragment of the *Streptomyces* plasmid SCP2* may also carry information for partitioning of the plasmid between dividing cells. However, definitive studies of the segregation of plasmids are difficult in a mycelial organism which

may contain several chromosomal genomes per cell and which undergoes a complex process of differentiation and sporulation.

The transfer properties of the plasmid vectors and their abilities to promote chromosomal recombination were also studied. Examination of the fertility properties of each of the SCP2* derivatives (Table 2) indicates that SCP2* increases the level of chromosomal recombination approximately 10^5 -fold over that observed in the absence of any plasmids and that this plasmid is transferred at a frequency of 100% to the recipient culture⁶. Each of the derivatives of this plasmid had fertility and transfer properties similar to those of the parent, indicating that the fertility determinants of SCP2* as well as the transfer functions are located in the region of the plasmid genome covered by the *Pst*I-A and *Bam*HI-A fragments.

In experiments analogous to those described above, isolates were obtained from the *S. lividans* transformation mixture (Fig. 2) that elicited lethal zygosis against *S. lividans* 1326. Plasmid DNA extracted from these clones was analysed by digestion with *Pst*I or *Eco*RI endonucleases and shown to consist of the larger *Pst*I fragment of SLP1.2. This derivative, designated pSLP101, contains unique *Pst*I and *Bam*HI sites located in a non-essential region of the plasmid; one of the sites for both *Hind*III and *Sal*I also occurs in this region. This plasmid is potentially useful for the cloning of DNA fragments produced by any of these four enzymes.

Conclusions

The findings reported here demonstrate the practicality of DNA cloning and heterospecific expression of cloned genes in *Streptomyces*. Because transformation of *Streptomyces* protoplasts is highly efficient, it is possible to clone genes from the chromosome of donor DNA preparations. One of the vectors we have constructed (pSCP111) replicates and expresses resistance to methylenomycin in at least three different *Streptomyces* species, each of which produces at least one antibiotic (refs 11, 19, 20 and M.B., unpublished). Each vector contains an antibiotic resistance determinant that is directly and easily selectable in most *Streptomyces* species and has single cleavage sites in non-essential regions for several different restriction endonucleases, some of which yield DNA termini especially useful for cloning a wide variety of differently generated fragments. For example, DNA fragments generated by *Bgl*II, *Bcl*I, *Sau*3A or *Mbo*II have protruding 5' ends identical to those produced by *Bam*HI and can be cloned at the *Bam*HI site of these vectors. In addition, fragments of chromosomal DNA produced by random shearing can be introduced into the *Pst*I cleavage site by a dG-dC homopolymeric tailing procedure (for example, see ref. 21).

The ability to clone genes in *Streptomyces* should prove valuable in the analysis and, ultimately, the manipulation of antibiotic synthetic pathways of these organisms²². Such manipulation may enable new antibiotics to be produced by the introduction of exogenously derived enzymes that modify pre-existing antibiotics or lead to the synthesis of hybrid antibiotic molecules. In addition, the cloning on multi-copy plasmids, such as pSLP101, of genes involved in rate-limiting biosynthetic steps may prove useful in increasing the yield of antibiotics produced by *Streptomyces* species.

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²H NMR study of molecular motion in collagen fibrils

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Collagen was labelled through tissue culture with [3,3,3-*d*₃]alanine. ²H NMR spectra were obtained of the labelled collagen as fibrils and in solution using the quadrupolar echo technique for solids. The ²H NMR data were analysed in terms of a model for reorientation in which the molecule is considered to jump between two orientations in a time which is short compared to the residence time in each site, and short compared to $(\Delta\nu_q)^{-1}$. The best fit of the data indicates that the collagen molecule in the fibrils experiences reorientation about its long axis over an angular range of ~30–40°. The *T*₂ for [3,3,3-*d*₃]alanine-labelled collagen fibrils is estimated to be ~110 μs.

CURRENT information about the spatial distribution of amino acids in the collagen molecule has mainly come from X-ray fibre diffraction and model building^{1–4}. These methods, by their nature, lead to a concept of a static collagen molecule in the fibril. We report here ²H NMR results which, in conjunction with previous ¹³C NMR results^{5,6}, require a motionally dynamic model for the collagen molecule in the fibril. We have used solid state ²H NMR of [3,3,3-*d*₃]alanine-labelled collagen to obtain information about molecular motion of the collagen peptide backbone in the fibrils, and to obtain preliminary information on the orientation of the C^α–C^β bond axis of alanine residues in the collagen molecule. In addition, we present equations which allow a simple calculation of the ²H NMR lineshape in the presence of motion. These results provide strong evidence that the peptide backbone in collagen fibrils undergoes rapid ($\tau_c \sim 10^{-7}$ s) motion about the long axis of the molecule and that this motion takes place over an ~30–40° range in azimuthal angle. To our knowledge, this work represents the first application of solid state ²H NMR as a probe for the molecular dynamics of a labelled protein, although this technique has been used very successfully in the membrane field⁷.

Solid state ²H NMR is well suited for the study of molecular motion for several reasons. First, relaxation in ²H NMR is dominated by the quadrupolar interaction, and therefore no questions arise concerning the source of the relaxation mechanism in ²H NMR. Second, the deuterium field gradient tensor for most C–D bonds is generally axially symmetric with the unique principal axis along the C–D bond direction⁸, which makes spectral interpretation straightforward. Third, ²H NMR quadrupolar powder patterns are sensitive to motions which have correlation times $\tau \approx (\Delta\nu_q)^{-1}$, where $\Delta\nu_q$ is the deuterium quadrupole splitting⁷. Fourth, the low natural abundance (0.016%) of deuterium virtually eliminates background signals due to unlabelled material in natural abundance.

Labelling of collagen and measurement of spectra

Lathyrus collagen was labelled with [3,3,3-*d*₃]alanine through chick calvaria culture by previously described methods^{5,6}. Characterisation of the protein by amino acid analysis indicated that it was pure collagen⁹. Optical rotatory dispersion at 313 nm (ref. 10) established that the entire collagen sample was in native form on completion of the ²H NMR experiments. The per cent

incorporation of deuterated alanine in the protein was determined by chemical ionisation gas chromatography–mass spectroscopy of *N*-acetyl methyl ester derivatives^{5,6} of the enzymatically hydrolysed protein. A small amount of proton exchange occurs at alanine during acidic hydrolysis of proteins¹¹. An enzymatic hydrolysis procedure (Pronase and aminopeptidase M) was therefore developed for collagen to avoid artefacts introduced by acid-catalysed exchange on the determination of the per cent incorporation of ²H. Radiotracer analysis (both ³H and ¹⁴C) indicated that no amino acids other than alanine were labelled during biosynthesis. The labelled collagen was studied by ²H NMR both in solution (~10 mg ml⁻¹ in 0.1 M acetic acid) and as fibrils (in equilibrium with 0.02 M Na₂HPO₄). ²H NMR spectra were obtained using a 'home-built' spectrometer operating at 5.2 T (33.78 MHz for ²H). The $\pi/2$ pulse was 5 μs. A solid echo pulse sequence ($90^\circ_{\pm x} - t_1 - 90^\circ_y - t_2 - T$) (refs 12–14) was used for all experiments to preserve the inhomogeneously broadened portion of the NMR signal, which is normally lost through spectrometer dead time. The phase of the first 90° pulse was shifted by 180° on alternate scans to reduce artefacts due to coil ringing and imperfect 90° pulses. All data were acquired in quadrature (2,000 points per channel) with a sampling rate of 5 μs per point. Signal distortion due to pulse power fall-off was less than 10% over the range ±40 kHz, and the lineshapes presented here were not corrected. A control ²H NMR spectrum of unlabelled collagen was run for every ²H NMR spectrum of labelled collagen discussed here. This ensured that the portion of the signal arising from [3,3,3-*d*₃]alanine-labelled collagen and the portion arising from deuterated water in natural abundance could be assigned with confidence.

Analysis of the spectra

The ²H NMR spectra for L-[3,3,3-*d*₃]alanine and for various alanine-labelled collagen samples are presented in Fig. 1. As a powdered amino acid, L-[3,3,3-*d*₃]alanine exhibits the classical ²H NMR powder pattern (Fig. 1*a*). The observed quadrupolar splitting ($\Delta\nu_q = (3e^2qQ/4h)(3\cos^2\theta' - 1)/2$) of 38.8 kHz (Table 1) shows that the static quadrupolar coupling constant ($e^2qQ/h \approx 165$ kHz) has been averaged to approximately one-third of its value by rapid rotation of the methyl group¹⁵. Frozen [3,3,3-*d*₃]alanine-labelled collagen fibrils (Fig. 1*b*) exhibit a ²H NMR lineshape similar to that of the powdered amino acid. The

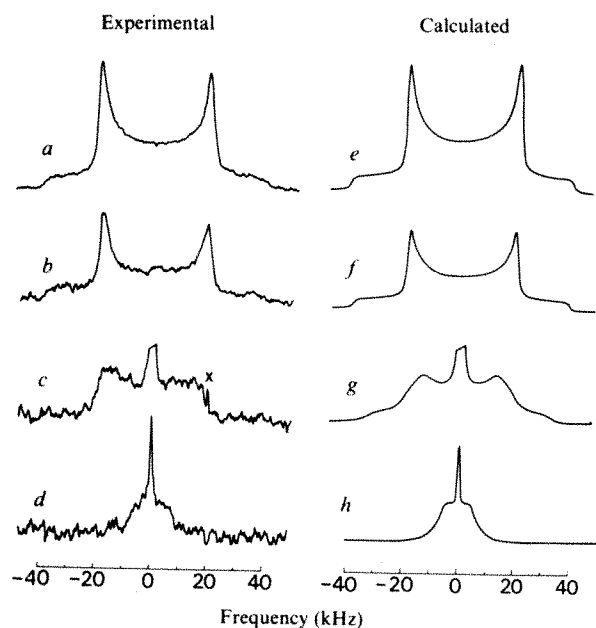


Fig. 1 Experimental ^2H NMR spectra for $[3,3,3\text{-d}_3]$ alanine and $[3,3,3\text{-d}_3]$ alanine-labelled collagen. These spectra were obtained in quadrature using the solid echo pulse sequence ($90^\circ_{\text{xx}} - t_1 - 90^\circ_{\text{yy}} - t_2 - T$) with t_1 and t_2 set at 50 and 59 μs , respectively. *a*, $[3,3,3\text{-d}_3]$ Alanine as a polycrystalline amino acid; 256 accumulations, 0.25 s repetition rate, 18 $^\circ\text{C}$; *b*, $[3,3,3\text{-d}_3]$ alanine-labelled collagen fibrils, 20 mg in equilibrium with excess Na_2HPO_4 ; 2×10^5 accumulations, 0.25 s repetition rate, -18 $^\circ\text{C}$; *c*, the same as in *b*, except at 18 $^\circ\text{C}$; *d*, $[3,3,3\text{-d}_3]$ alanine-labelled collagen in solution, ~ 10 mg/ml in about 0.4 ml 0.1 M acetic acid in deuterium-depleted water; 2.5×10^5 accumulations, 0.1 s repetition rate, 18 $^\circ\text{C}$; *e-h*: calculated spectra for $[3,3,3\text{-d}_3]$ alanine and for $[3,3,3\text{-d}_3]$ alanine-labelled collagen. Lorentzian line-broadening was used in all cases; *e*, calculated spectrum of polycrystalline $[3,3,3\text{-d}_3]$ alanine; $\Delta\nu_q = 38.8$ kHz, line-broadening = 1.3 kHz; *f*, Calculated spectrum of frozen $[3,3,3\text{-d}_3]$ alanine-labelled collagen fibrils; $\Delta\nu_q = 37.3$ kHz, line-broadening = 1.5 kHz; *g*, Calculated spectrum of $[3,3,3\text{-d}_3]$ alanine-labelled collagen fibrils at 18 $^\circ\text{C}$. The alanine $\text{C}^\alpha\text{-C}^\beta$ bond axes are assumed to undergo jump diffusion between two equally populated sites. The angles θ between the $\text{C}^\alpha\text{-C}^\beta$ bond axes and the helix axis are 69° and 85° (Fig. 2*a, b*). The angle 2δ through which the projected alanyl $\text{C}^\alpha\text{-C}^\beta$ bond axis is carried by reorientation of the peptide backbone is 40° (Fig. 2*b, c*). The spectra calculated for $\theta = 69^\circ$ and $\theta = 85^\circ$ were summed with equal weight; $\Delta\nu_q = 37.3$ kHz, line-broadening = 4.1 kHz. A Lorentzian signal with 600 Hz linewidth was included in the centre of the spectrum to simulate the natural abundance signal from water. *h*, Calculated spectrum of $[3,3,3\text{-d}_3]$ alanine-labelled collagen in solution. Reorientation of the alanyl $\text{C}^\alpha\text{-C}^\beta$ bond axis about the long axis of the helix has averaged the crystalline 37.3 kHz quadrupolar splitting to ~ 10 kHz. Line-broadening = 4.1 kHz.

quadrupolar splitting for the frozen fibrils is about 3% smaller than for the labelled amino acid (Table 1). This result may arise from a small amount of molecular motion (in addition to methyl rotation) which occurs in the fibrils at -18 $^\circ\text{C}$, or may reflect a slightly smaller quadrupolar coupling constant for alanine in a polypeptide.

At +18 $^\circ\text{C}$, $[3,3,3\text{-d}_3]$ alanine-labelled collagen fibrils exhibit a ^2H NMR spectrum in which about 60% of the signal intensity is lost and in which the singularities are no longer well defined (Fig. 1*c*; Table 1). To establish that the observed lineshape (Fig. 1*c*) was representative of the entire sample, a spectrum was obtained at the shortest values of t_1 and t_2 which were consistent with recovery of the spectrometer (30 and 39 μs , respectively). This spectrum contained more intensity, but was identical in shape (within uncertainty due to baseline distortion) to the spectra obtained at longer t_1 and t_2 values. The apparent reduction of the quadrupolar splitting and the loss of signal intensity

through homogeneous T_2 processes¹⁶ observed in Fig. 1*c* imply reorientation of the alanine methyl in collagen fibrils in addition to 3-fold rotation. Motion of the alanine methyl group (the $\text{C}^\alpha\text{-C}^\beta$ bond axis) requires reorientation of the peptide backbone in collagen because alanine contains no additional bonds about which reorientation can take place.

The ^2H NMR spectrum of $[3,3,3\text{-d}_3]$ alanine-labelled collagen in solution shows a further reduction of the spectral width (Fig. 1*d*; Table 1). In solution, collagen behaves as a rod-like monomer of molecular weight 285,000 and dimensions of $\sim 1.5 \times 300$ nm¹⁷. The molecule in solution can undergo free rotation about its long axis, ($R_1 \sim 10^7$ s⁻¹), whereas end-over-end rotation is slow ($R_2 \sim 10^2$ s⁻¹) and is restricted at these concentrations due to occupied volume considerations^{5,6}. Accordingly, the ~ 37 -kHz quadrupolar splitting observed for the frozen labelled fibrils would be expected to collapse by a factor of $(1-3\cos^2\theta)/2$ in the presence of free rotation about the long axis of the collagen molecule. Here, θ is the angle formed between the $\text{C}^\alpha\text{-C}^\beta$ bond axis and the long axis of the molecule (Fig. 2*a*). The observed quadrupolar splitting for labelled collagen in solution is ~ 10 kHz, which requires that θ be $\sim 70^\circ$. An angle of $\sim 70^\circ$ for θ is in good agreement with the $\text{C}^\alpha\text{-C}^\beta$ bond angles predicted by various models for the collagen helix^{1,3,18} which place θ between 60° and 90° . Comparison of the ^2H NMR intensity from the labelled sample with the intensity from the same amount of unlabelled collagen (deuterium-depleted water used in both cases) indicates that about half the intensity of the sharp peak in Fig. 1*d* arises from the alanine-labelled protein in solution and that the rest is due to deuterated water in natural abundance. Previous ^{13}C NMR experiments^{5,6},

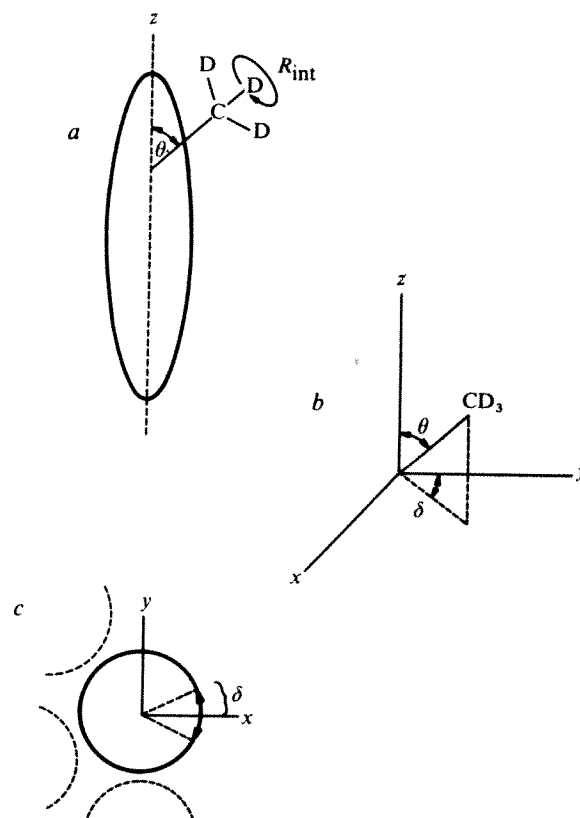


Fig. 2 Schematic representation of the alanine methyl group attached to the collagen helix. *a*, The collagen helix considered as an ellipsoid. The $\text{C}^\alpha\text{-C}^\beta$ bond of alanine makes the angle θ with the long axis of the helix; *b*, the methyl group of alanine, which makes an angle θ with respect to the helix axis (the z axis), undergoes reorientation between two sites separated by an angle 2δ ; *c*, Cross-section of collagen molecules assembled into fibrils. The molecules undergo reorientation about the long axis of the helix over an azimuthal angle of 2δ .

Table 1 Quadrupolar splitting ($\Delta\nu_q$) for [3,3,3- d_3]alanine and [3,3,3- d_3]alanine-labelled collagen fibrils

Sample	$\Delta\nu_q^*$ (kHz)
[3,3,3- d_3]Alanine	38.8
[3,3,3- d_3]Alanine-labelled collagen fibrils in 0.02 M Na_2HPO_4	
-18°C	37.3
+18°C	~30†
lyophilised	38
[3,3,3- d_3]Alanine-labelled collagen in solution	~10†

* $\Delta\nu_q = \frac{3e^2qQ}{4h} (3 \cos^2 \theta' - 1)/2$; θ' is the angle made by the $\text{C}^\alpha\text{-C}^\beta$ -D and $\text{C}^\alpha\text{-C}^\beta$ bond axes.

†Axially asymmetric pattern; value reported is the frequency difference between maxima in the calculated spectrum and corresponds to the apparent splitting.

in conjunction with the present sample characterisation, indicate that it is unlikely that this peak is due to a small amount of denatured protein. However, the sharp component may be due to some alanine $\text{C}^\alpha\text{-C}^\beta$ bond axes which make the 'magic angle' (54.7°) with respect to the helix axis, or to the 3% of the alanine residues which reside in the non-helical termini of the molecule².

The ^2H NMR spectra of labelled collagen fibrils were analysed by matching the experimental lineshapes with lineshapes calculated in the presence of molecular reorientation. In the motional model utilised for these calculations, the molecule is considered to jump between two orientations in a time which is much less than the residence time in each of the two sites¹⁹ and much less than $(\Delta\nu_q)^{-1}$.

The assumed motion of the collagen backbone makes the alanine $\text{C}^\alpha\text{-C}^\beta$ bond axis jump between two orientations, labelled 1 and 2 in Fig. 3a. As a consequence of the motion, the observed powder pattern will not generally be axially symmetric. Using Fig. 3a, one can determine the principal axis system (x, y, z) and the frequencies ($\omega_x, \omega_y, \omega_z$) corresponding to the principal values of the motionally averaged field gradient tensor as follows. For clarity we consider the powder pattern corresponding to the transition for which $\omega_\perp \leq \omega \leq \omega_\parallel$ (see Fig. 3b). When H_0 (the applied field) is normal to the 1, 2 plane, $\omega = \omega_\perp$ in both the 1 and 2 orientations. Since this is the minimum value possible for ω , x must be normal to the 1, 2 plane (we use the convention $\omega_x \leq \omega_y \leq \omega_z$) and $\omega_x = \omega_\perp$. The remaining principal axes, y and z , are perpendicular to x and are thus in the 1, 2 plane. When H_0 is in this plane:

$$\begin{aligned}\omega &= 0.5[\omega_\parallel(\cos^2 \theta_{1H} + \cos^2 \theta_{2H}) \\ &\quad + \omega_\perp(\sin^2 \theta_{1H} + \sin^2 \theta_{2H})] \\ \omega &= -0.5\omega_\perp[3 \cos^2 \theta_{1H} + 3 \cos^2(\gamma_{12} - \theta_{1H}) - 2]\end{aligned}$$

where θ_{1H} and θ_{2H} are the respective angles made by H_0 with 1 and 2. When H_0 lies along z , ω is maximal, and solving the equation $d\omega/d\theta_{1H} = 0$ shows that z bisects γ_{12} when γ_{12} is in the first or fourth quadrants, and that z is perpendicular to the bisector of γ_{12} when $90^\circ \leq \gamma_{12} \leq 270^\circ$. The y axis is normal to the xz plane. The frequencies corresponding to the field along the principal axes are

$$\begin{aligned}\omega_x &= \omega_\perp \\ \omega_y &= -\omega_\perp[3 \sin^2(\gamma_{12}/2) - 1] \\ \omega_z &= -\omega_\perp[3 \cos^2(\gamma_{12}/2) - 1]\end{aligned}$$

These results apply for γ_{12} in the first or fourth quadrant. When $90^\circ \leq \gamma_{12} \leq 270^\circ$, the expressions for ω_y and ω_z are interchanged. Note that an axially symmetric pattern occurs only when $\gamma_{12} = 0, 90$ or 270° (Fig. 3b), and a pattern having $\eta = 1$ occurs whenever ω_y equals zero (Fig. 3c). The correspondence

between γ_{12} and the angles (θ, δ) which describe the reorientation of the molecule is: $\cos \gamma_{12} = \cos^2 \theta + \sin^2 \theta \cos^2 2\delta$.

The relevant angles which describe the orientation of the alanine methyl group in the collagen molecule are shown in Fig. 2. The angle formed by the alanine $\text{C}^\alpha\text{-C}^\beta$ bond axis and the long axis of the collagen helix is designated as θ (Fig. 2a,b). The angle δ is half of the angle through which the alanine $\text{C}^\alpha\text{-C}^\beta$ bond is carried by reorientation of the peptide backbone (Fig. 2b,c).

The various collagen models predict that the angle which the $\text{C}^\alpha\text{-C}^\beta$ bond makes with the helix axis depends on whether the considered amino acid occurs in the X or Y position of the Gly-X-Y triplet sequence^{1,3,18}. Accordingly, the lineshapes from two representative values of θ (85° and 69°) were summed with equal weight (alanine is distributed equally in the X and Y positions in collagen) for each calculated spectrum. The angle δ and the line broadening were varied until the best match of the lineshape was obtained.

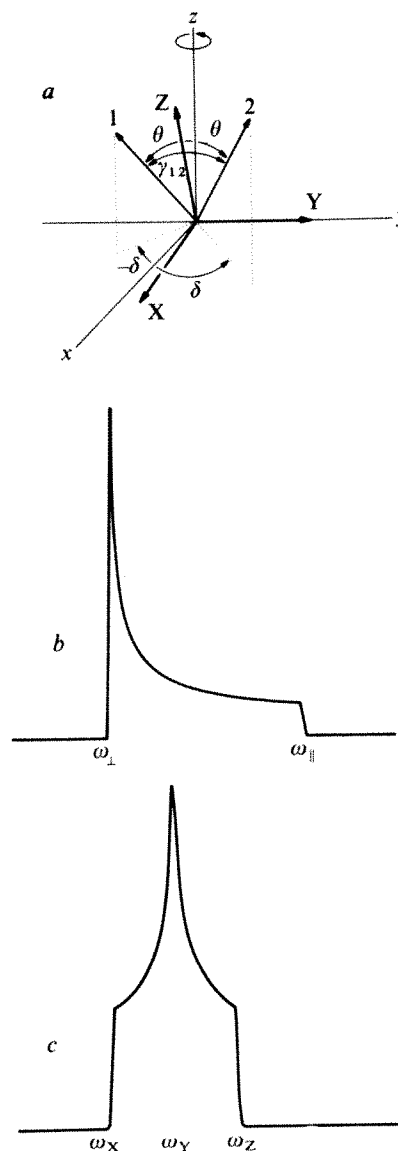


Fig. 3 a, The geometrical construction used to deduce ω_x, ω_y and ω_z in the presence of two-site jump reorientation. 1 and 2 refer to the two orientations between which the alanine $\text{C}^\alpha\text{-C}^\beta$ bond axis is considered to jump. The angles θ, δ , and γ_{12} are described in the text; b, a theoretical axially symmetric powder pattern (one transition for the case when $\eta = 0$); c, the theoretical pattern expected when $\eta = 1$ (when $\omega_y = 0$). In the presence of line-broadening, this pattern would lose definition in the edges and have an overall tent-like appearance.

Implications for models of collagen

The best fit of the data (Fig. 1c,g) indicates that the collagen molecule in the fibril undergoes reorientation over a $\sim 30\text{--}40^\circ$ range in azimuthal angle (that is, $2\delta \approx 30\text{--}40^\circ$; Fig. 2c). This angle is in good agreement with an estimate obtained from previous ^{13}C NMR studies^{5,20} in which the minimum angle, 2δ , consistent with the ^{13}C T_1 data, was $\sim 30^\circ$. The assumption used in the calculations of the ^2H NMR lineshapes (that the jump rate is much larger than $\Delta\nu_Q$) is consistent with the reorientation rate of about 10^7 s^{-1} , derived from the ^{13}C studies^{5,6,20}. A line-broadening of $\sim 4\text{ kHz}$ is required to fit these ^2H NMR data. A portion of this line-broadening ($\sim 3\text{ kHz}$) can be explained by the T_2 , which we estimate to be $\sim 110\text{ }\mu\text{s}$. The rest of the line-broadening is attributed to D-H dipolar interactions. Mass spectroscopic analysis showed that of the alanine residues which were labelled, about half contained three deuterons, one quarter

contained two deuterons, and one quarter contained one deuteron. Note that the partial loss of deuterons from alanine must occur during biosynthesis as mass spectroscopy indicates that $\sim 95\%$ of the starting $[3,3,3\text{-d}_3]\text{alanine}$ and $\sim 95\%$ of the control labelled alanine for the enzymatic hydrolysis contained three deuterons. A second moment calculation⁸ indicates that the static D-H interaction between a deuteron and a single proton on the same alanine methyl carbon²¹ causes an average line-broadening of $\sim 3.5\text{ kHz}$. Three-fold rotation of the methyl group would average this to $\sim 1.7\text{ kHz}$.

The ^2H NMR results presented here, in conjunction with earlier ^{13}C NMR results^{5,6,20}, provide strong evidence that the peptide backbone in collagen fibrils undergoes rapid ($\tau_c \leq 10^{-7}\text{ s}$) anisotropic motion about the long axis of the molecule. The range in azimuthal angle over which this motion takes place is estimated to be $30\text{--}40^\circ$.

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LETTERS

The γ -ray background and the age of protogalaxy formation

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The diffuse γ -ray background which is implied by the current models of protogalaxy formation is considered here in an attempt to set limits to the redshift z_i of that event. This is obtained by observing that cosmic rays from supernovae exploding in protogalaxies produce an isotropic γ -background in excess of the observed values if protogalaxies form too early. Without any special assumptions, the constraints on z_i turn out to be more significant than any other limit previously reported. In fact, for z_i to be >100 , one must postulate either a very low cosmic ray production in supernovae, or a very low density of intergalactic gas, that is, $<10^{-10}\text{ cm}^{-3}$, or both.

We consider only specific cases which set the age of galaxy formation at a redshift $z_i \leq 100$. In this case, even for a 'dense interstellar gas universe' ($n_0 \approx 10^{-5}\text{ particles cm}^{-3}$), all proton and photon absorption and degradation effects become minimal, and we can consider the simplified model of γ -ray propagation in a vacuum by thin target scattering.

In contrast, if $z_i \geq 100$, a different model, which can be simplified according to Cavallo and Rees¹, and Cavallo and Pacini² should be used. However, the predictions of this second approach depend critically on the precise shape of the 'fireball' photon spectrum in the neighbourhood of 0.5 MeV, a shape that has not yet been reported. Therefore, we shall only consider the simpler case $z_i \leq 100$, which already gives some interesting results.

The circumstances of protogalaxy formation are well known: at a given z_i , appropriate light element gas clouds collapse and

fragment, producing first generation stars. These stars will evolve according to canonical models, and will manufacture the heavier elements which will then be found in the second generation stars. Supernova explosions will expel and mix these heavier elements in the interstellar gas, and their abundances can presently be observed.

Note that supernovae are also thought to be the source of cosmic ray energy. Cosmic rays in their turn will produce γ -rays through the decay of neutral mesons produced in collisions with the interstellar gas. Current views, therefore, suggest that when protogalaxies formed light gas was converted into heavier elements and cosmic rays. Such a conversion into high energy particles might be approximated by a burst of cosmic rays taking place at a certain time z_b , in general quite close to z_i . In turn, such a burst might be incompatible with the presently observed γ -ray flux. To calculate a minimum value for the γ -ray background, we assume that cosmic rays directly or indirectly receiving their energy from supernova explosions interact mainly with the intergalactic gas. If the galaxies or, worse, the remnants, were opaque to cosmic rays, the energy conversion into γ -rays would be much more efficient, and our limits on z_i would thus be significantly lowered. Similarly, our results would be strengthened if the origin of the γ -background were not due to the mechanisms considered here.

The relevant computations go back to Stecker³ and Cavallo⁴, who obtained substantially similar results. The differential flux observed at $z = 0$ is given by

$$I_\gamma(E_\gamma) = cn_0 H_0^{-1} \int_0^{z_b} G_\gamma(E_\gamma; 1+z) \times (I(z)/I_0)(1+z)^{-5/2} dz \quad \text{photons cm}^{-2} \Delta E^{-1} \quad (1)$$

Here we have assumed that the cosmic ray flux $I(z)$ has the same energy dependence as I_0 , the presently observed galactic cosmic ray flux; moreover, an Einstein-de Sitter model has been adopted. The function $G_\gamma(E)$ is the differential specific emissivity produced by collisions of the galactic cosmic ray flux with the

interstellar gas in a 1-cm pathlength. One can show that, for $E_{\gamma e} = E_{\gamma o}(1+z)$ (subscripts e and o refer to emitted and observed respectively) the differential emissivity becomes: $G_k(E; 1+z) = (1+z)G_k[(1+z)E_{\gamma}]$. For a burst model, one has³:

$$I(z)/I_g = A(1+z)^3(1+z)^{3/2}(1+z_b)^{-3/2} \quad (2)$$

where one should observe the normalisation $I_o/I_g = A(1+z_b)^{-3/2}$. Thus the integral becomes:

$$I_{\gamma}(E_{\gamma}) = cn_0 H_0^{-1} A(1+z_b)^{-3/2} \int_0^{z_b} G_k[E_{\gamma}(1+z)](1+z)^3 dz \quad (3)$$

An easy comparison with experiment can be made by integrating the flux for $E_{\gamma} \geq 100$ MeV. If $y = E_{\gamma}(1+z)$, we obtain:

$$I_{\gamma}(E_{\gamma} > 100 \text{ MeV}) = cn_0 H_0^{-1} A(1+z_b)^{-3/2} \int_0^{z_b} (1+z)^2 dz \times \int_{100 \text{ MeV}/(1+z)}^{\infty} G_k(y) dy \quad (4)$$

Above 100 MeV and for $z \geq 3$ (a value comparable with the maximum z observed in QSOs) a good approximation is the relationship

$$\int_{(1+z)100 \text{ MeV}}^{\infty} G_k(y) dy = 2 \times 10^{-25} (1+z)^{-3/2} \quad (5)$$

Therefore:

$$I_{\gamma}(E_{\gamma} > 100 \text{ MeV}) = 2 \times 10^{-25} cn_0 H_0^{-1} (I_o/I_g)(2/3)[(1+z_b)^{3/2} - 1] \quad (6a)$$

$$\approx 1.3 \times 10^{-25} cn_0 H_0^{-1} (I_o/I_g)(1+z_b)^{3/2} \quad \text{for } z_b \gg 1 \quad (6b)$$

Equation (6b), divided by 4π , may be compared with the flux 1×10^{-5} photons $(\text{cm}^2 \text{s sr})^{-1}$, given in ref. 5, smaller by a factor of 10 than the flux adopted by Stecker³. The desired result is, therefore,

$$I_o/I_g \leq [1 \times 10^{-2}](1+z_b)^{-3/2} h_{100} n_5^{-1} \quad (7)$$

where $h_{100} = H_0/(100 \text{ km s}^{-1} \text{ Mpc}^{-1})$ and $n_5 = n_0/(10^{-5})$. Equation (7) gives a good approximation to the values given in ref. 3, apart from the factor of 10 mentioned above. The theory of star formation gives some indication of the value of I_o/I_g . Our model assumes that a fraction of $\sim 2.8 \times 10^{-6} E_{50}$ protons per supernova of at least $6 M_{\odot}$ goes into cosmic rays. Here, E_{50} is the total energy emitted in cosmic rays of average energy 3 GeV, measured in units of 10^{50} erg. If all matter had gone through the supernova stage, the ratio I_o/I_g would be $\sim E_{50}/4$ at present. In fact, only an appropriate fraction $f(z_b)$ of all matter went into supernovae, specifically, only those stars whose mass exceeds $\sim 6 M_{\odot}$. The general result is therefore:

$$f(z_b)(1+z_b)^{3/2} \leq [1 \times 10^{-2}](E_{50}/4)^{-1} h_{100} n_5^{-1} \quad (8)$$

Here a method of computing $f(z_b)$ is used based on current fragmentation models, which predict a specific minimum mass M_{ff} for the ultimate fragments. M_{ff} depends on the background temperature according to the law⁶:

$$M_{\text{ff}} = 1.54 \times 10^{-3} (m_{\text{H}}/m)^2 T_b^2 (k_{\text{pt}}/k_0) M_{\odot} \quad (9)$$

with $T_b = 2.7(1+z)\text{K}$; m_{H} and m are the mass of the hydrogen atom and of the typical atom of the fragmenting gas cloud, k_{pt} is the final Planck mean opacity per unit mass, and k_0 is the opacity due to free electron scattering in ionised hydrogen. The existence of such a temperature dependent minimum mass ensures us that the earlier the protogalaxies formed, the more complete was the conversion of light gas into heavier elements and cosmic rays.

Assuming a Salpeter⁷ initial mass function $\psi(m) = K m^{-2.3}$, we can write:

$$f(z_b) = \int_{6M_{\odot}}^{\infty} m\psi(m) dm / \left(\int_{M_{\text{ff}}}^{\infty} m\psi(m) dm \right) \quad (10)$$

which becomes:

$$f(z_b) = \begin{cases} 0.15(1+z_b)^{0.6} & \text{for } z_b \leq 22.12 \\ 1 & \text{for } z_b \geq 22.12 \end{cases} \quad (11a)$$

$$(11b)$$

Consequently one should satisfy the relationship:

$$0.27 \geq (1+z_b)^{21/10} E_{50} n_5 h_{100}^{-1} \quad \text{for } z_b \leq 22 \quad (12a)$$

$$4 \times 10^{-2} \geq (1+z_b)^{3/2} E_{50} n_5 h_{100}^{-1} \quad \text{for } z_b \geq 22 \quad (12b)$$

Thus, in general to have $z_b \geq 3$, one needs $\epsilon E_{50} n_5 h_{100}^{-1} \leq 1.5 \times 10^{-2}$ where ϵ is the efficiency of protogalaxy formation. The current value $\epsilon = 1$, and we conclude that in this framework ($n = 10^{-5} \text{ cm}^{-3}$) there is no chance of finding an appropriate z_b . The only effective possibility is that of lowering n_0 to, say, 10^{-7} cm^{-3} . In this case we would obtain:

$$1+z_b \approx 4.8 (E_{50} n_7 h_{100}^{-1})^{-10/21} \quad (13)$$

and we suggest this value of $z_b \approx 4$ as a possible burst age. An intergalactic gas density of $< 10^{-10}$ is needed to achieve $z_b \geq 100$. Of course, this does not mean that we favour an open universe: we can only say that the intergalactic gas is likely to give an irrelevant contribution to the average matter density of the universe.

The foregoing results depend on some common assumptions which we now examine.

(1) The validity of equations (9) and (10): In fact, the $(1+z)$ dependence in equation (9) is weakened in equation (10), and would be practically ineffective for a $\psi(m) = K m^{-2}$ power law. Thus, equation (9) does not strongly affect our results. The possibility that the initial mass distribution is appreciably different from the Salpeter function is more important. One must, however, observe that supernovae are needed for manufacturing heavy elements, without producing too many of them. There are two alternatives: (i) the initial distribution of stars, although non-Salpeter-like, mostly consists of massive stars; (ii) the initial distribution mostly consists of low mass stars. The first alternative, which supports our conclusions, has the risk of producing too many heavy elements, but in the second alternative the minimum mass should be such that supernovae can produce a sufficient amount of heavy elements, without leaving behind too many first generation stars. Therefore, the assumption implicit in equation (10) is not really critical.

(2) The validity of the assumption that each supernova produces 10^{50} erg in cosmic rays. At first, this might seem to be a very uncertain figure, as it depends on two poorly known parameters—the explosion rate and the cosmic ray diffusion coefficient. However, estimates of the total particle content for many remnants⁸, and considerations of the kinetic energy input in remnants, using some equipartition assumptions⁹, all indicate a rather restricted range of values around and perhaps greater than $E_{50} = 1$. A finer point is to consider the fate of the protons thus produced. It is commonly assumed that the energy requirements for cosmic rays are met by supernovae: the cosmic rays might directly come from the first stages of the explosion (as in the Colgate¹⁰ model) or the energy of supernovae might be used to accelerate cosmic rays by interstellar shocks. In both cases our results will stand.

(3) Finally, protogalaxy formation might have a small efficiency. Hogan and Layzer¹⁰ made similar assumptions to explain the microwave and X-ray background, but they had to resort to a high yield of relativistic electrons per supernova ($3-7 \times 10^{48}$ erg). The above criticisms also apply to their model, but our results on z_b turn out to be more restrictive, while giving information about the intergalactic gas density which the previous authors could not consider. Our stricter limits, apart from minor technical details, such as the assumption of an Einstein-de

Sitter universe with a slightly higher conversion of particles into relativistic cosmic rays, result from the following: (1) the multiplicity of meson production is >1 and increases with energy; (2) there are about 100 times more protons than electrons in cosmic rays; (3) most important of all, the energy content of the diffuse X-ray background exceeds by about two orders of magnitude the energy content in the isotropic γ -ray background.

Therefore we believe that γ -ray astronomy is still one of the most useful sources of information on the early universe.

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Inhibition of diffusion by the reflection effect in binary Hg–Mn stars

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The Hg–Mn stars constitute a sub-group of the peculiar A stars which have abundance anomalies in their atmospheres. Their distinctive properties are excesses of P, Mn, Ga, Y and Hg¹, slow rotation², a high frequency of double-lined spectroscopic binaries^{3,4}, and a lack of detectable magnetic fields⁵. It is widely believed that the abundance anomalies are at least partly due to the separation of elements by diffusion under the influence of gravitation and radiation pressure^{6,7}. For the diffusion mechanism to operate the atmosphere has to be extremely quiescent with circulation velocities $\leq 10^{-3} \text{ m s}^{-1}$. We point out here that in a spectroscopic binary system with similar components surface streaming velocities greatly in excess of this value are expected due to the mutual irradiation of the components.

The mutual irradiation creates temperature gradients over the respective surfaces of the binary components; hydrostatic equilibrium cannot be maintained and streaming away from the substellar points must occur. The mean effective temperature, T , over each illuminated hemisphere is given by

$$T^4 \sim T_0^4(1+f)$$

T_0 being the effective temperature of the averted hemispheres and f being the fraction of radiation from one star intercepted by the other. For a mean component separation a and a component radius R ,

$$f \sim 1/4(R/a)^2$$

The horizontal temperature gradient produces a horizontal pressure difference ΔP which drives a streaming motion with characteristic velocity v . Thus for a characteristic photospheric density ρ

$$\Delta P \sim \frac{1}{2}\rho v^2$$

which with the gas equation, and in the absence of a significant magnetic field and radiation losses, yields

$$v \sim 0.18 (\Delta T)^{1/2} \text{ km s}^{-1}$$

where

$$\Delta T = T - T_0 \sim T_0 f/4$$

(compare with ref. 8 where a coefficient 0.36 was derived by approximate application of the equation of motion).

The periods of known Hg–Mn spectroscopic binaries range from 3 to 560 days; 13 of the 22 known binaries have periods of less than 10 days, and eight of these are double-lined with mass ratios near unity^{3,4}. For a typical Hg–Mn binary with a period of 6 days and components of masses $\sim 6M_\odot$, $a \sim 2 \times 10^{10} \text{ m}$. With $R \sim 2.7 \times 10^9 \text{ m}$, $f = 4.6 \times 10^{-3}$. Taking $T_0 \sim 14,000 \text{ K}$, one finds $\Delta T \sim 16 \text{ K}$ and $v \sim 700 \text{ m s}^{-1}$. Radiative losses are likely to reduce v by a factor of $\sim 10^2$ (ref. 9). Thus the characteristic streaming velocity will be $\sim 7 \text{ m s}^{-1}$. This velocity is four orders of magnitude greater than those envisaged on the diffusion mechanism. The Reynolds number is $\geq 10^7$ and the streaming is turbulent. Then the ratio of mixing to diffusive time scales is simply the ratio of the respective velocities, and so the photospheric layers will mix on a time scale $\leq 10^{-4}$ of the segregation time for diffusion in a quiescent atmosphere.

The question arises whether a magnetic field might constrain the motion. Equating magnetic and kinetic energies,

$$H^2/8\pi \sim \frac{1}{2}\rho v^2$$

where H is the magnetic field strength. Hence if $\rho \sim 10^{-9} \text{ g cm}^{-3}$, $H \sim 0.08 \text{ G}$. The threshold of detection of the line-of-sight component of H is $\sim 200 \text{ G}$, and so a magnetic field might possibly constrain the stream motion. It is not clear whether turbulent motions will occur in an atmosphere dominated by a magnetic field. However, even if laminar flow and diffusion occur over restricted regions of the stellar surface, vertical mixing must still take place at the boundaries of these regions.

Synchronous rotation has been assumed in the foregoing discussion. In this case the streaming pattern would be stable, but mixing at the boundary between the illuminated and averted hemispheres might prevent the formation of a spectrum variable. However, measurements of projected rotational velocities⁴ show that Hg–Mn stars in short-period binaries often have rotational periods longer than the orbital periods. With non-synchronous rotation the illumination will affect the various parts of the stellar surface in turn. The flow pattern is quickly established, and so streaming motion will be periodically induced over the entire stellar surface.

The streaming will decrease very rapidly with the optical depth τ , and diffusion at sub-photospheric layers, say $\tau \geq 5$, is not precluded by the reflection effect. However, models accounting for the overabundances and isotopic anomalies of Hg in terms of diffusive segregation at high levels¹⁰ seem to be excluded; almost all Mn stars have large overabundances of Hg (refs 11, 12).

The abundance anomalies of Hg–Mn stars do not seem to be correlated with their measured rotational velocities¹³. Such a correlation might have been expected if all the anomalies were due to diffusion limited only by meridional circulation.

The only current alternatives to diffusion are accretion models involving supernova ejecta¹⁴ or planetary bodies¹⁵ and so on. Simple accretion may produce overabundances but not deficiencies. However, we have not excluded sub-photospheric diffusion, and this may account for some of the observed deficiencies. Thus the explanation of Hg–Mn star anomalies may lie in a combination of accretion and sub-photospheric diffusion mechanisms.

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Santa Catharina meteorite and phase composition of irradiated Fe–Ni Invar alloys

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The iron meteorite Santa Catharina is an ataxite that contains 35% Ni. Laboratory made Fe–Ni alloys of this composition are called Invar alloys, due to their low thermal expansivity near room temperature. The heterogeneous phase composition of the meteorite, which is apparent from a metallographic examination, has been confirmed by thermomagnetic measurements¹ and by more recent Mössbauer spectra and microprobe analysis². The nature and origin of the alloy phases in Santa Catharina have been the subject of recent investigations^{3,4} and we report here Mössbauer spectra and X-ray diffraction measurements which show that the phase composition of the meteorite is similar to that obtained by electron irradiation of Fe–Ni Invar alloys⁵.

The extensive phase segregation and radiation-induced ordering which occurs in electron irradiated Invar alloys have been observed by small angle neutron scattering⁶ and by Mössbauer spectroscopy⁵. This is illustrated in Fig. 1a, b. One of the phases is paramagnetic and causes the central peak of the spectrum (Fig. 1b), and the other phase is ferromagnetic and causes the magnetic hyperfine splitting of the Mössbauer spectrum. Computer fitting shows that the latter spectrum consists of two six-line spectra which have asymmetric shapes caused by the presence of an electric field gradient which superimposes a quadrupole splitting on the magnetic hyperfine pattern. Both spectra are due to the ordered Fe–Ni phase with the L10 superstructure^{7,8}. One of the two overlapping spectra has an hyperfine magnetic field $H_i = 288$ kG and a quadrupole splitting $\Delta E_Q = +0.23$ mm s⁻¹. This spectrum is caused by large crystallites of the ordered phase having their magnetic axes parallel to the axes of the electric field gradient⁹. Small crystallites may have their magnetic axes perpendicular to the axes of the electric field gradient and thus produce different Mössbauer parameters ($H_i = 310$ kG, $\Delta E_Q = -0.12$ mm s⁻¹).

Figure 1c shows the spectrum obtained with a thin section of the Santa Catharina meteorite showing a well-resolved asymmetric Mössbauer spectrum with a magnetic hyperfine field $H_i = 288$ kG, and quadrupole splitting $\Delta E_Q = +0.20$ mm s⁻¹ and a central peak corresponding to a paramagnetic phase. The Mössbauer parameters corresponding to the magnetic phase shows that this phase in the meteorite is composed only of large crystallites of the ordered alloy with the L10 superstructure, and all their magnetic axes are parallel to the electric field gradient axes.

We have also measured the Mössbauer spectra of the irradiated alloy and of the meteorite in other conditions, such as at low temperatures (4.2 K), with powder absorbers, under the influence of an applied magnetic field up to 50 kG; no obvious differences were observed between the samples.

In Fig. 2, the X-ray diffraction pattern obtained with the unirradiated alloy (35% Ni) is compared with the irradiated alloy and the meteorite sample. Superstructure L10 lines are detected in the irradiated alloy and in the meteorite but with much higher intensity. This corresponds to what was observed in the Mössbauer spectra, that is, that the degree of ordering is much larger in the meteorite compared with that obtained by irradiation of the alloy.

In Fig. 3, the thermal variation of the X-ray lattice parameters of the unirradiated alloy is compared with the irradiated alloy and the meteorite sample.

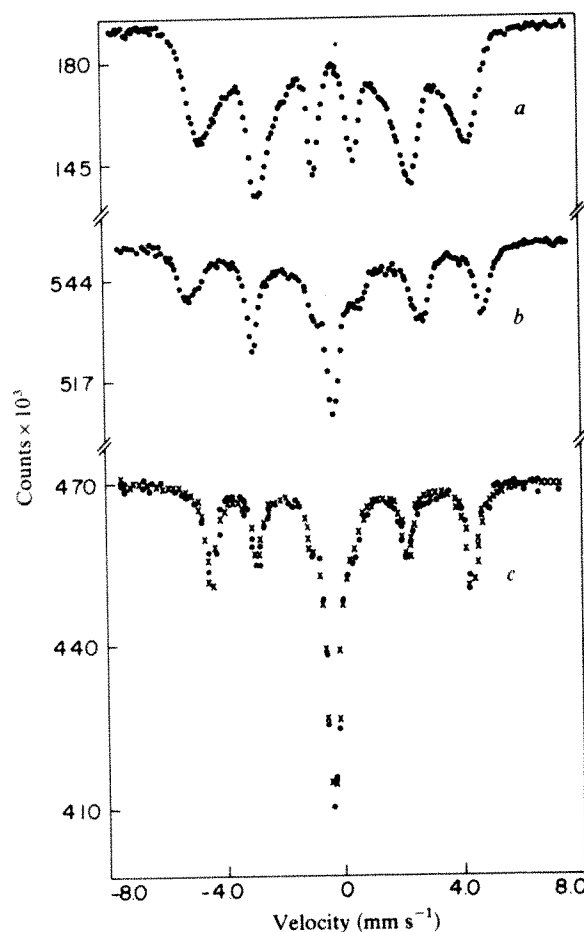


Fig. 1 Mössbauer absorption spectra recorded at room temperature with a ⁵⁷Co source in Rh of: a, Fe–Ni Invar alloy (35% Ni) before irradiation; b, after irradiation at 80 °C with 4×10^{19} 3-MeV electrons; c polished thin section of the Santa Catharina meteorite.

A large decrease of the lattice parameter, from 3.593 Å (Fig. 3a) to 3.582 Å (Fig. 3b) is observed on irradiating the Invar alloy. The two phases which are formed by irradiation have the same value for the lattice parameter. The Invar properties of the alloy are shown to disappear on irradiation by the linear variation of the lattice parameter with temperatures up to 300 °C. The same behaviour is observed with the sample of Santa Catharina, which exhibits a non-Invar thermal behaviour of the lattice parameter (Fig. 3c₁). The Invar property returns when the sample is heated at 800 °C for 24 h in a non-oxidising atmosphere (Fig. 3c₂).



Fig. 2 001 row of the reciprocal space observed with a single crystal diffractometer using the $K\alpha$ Co filtered radiation: *a*, Fe-Ni Invar alloy before irradiation; *b*, Fe-Ni Invar alloy after irradiation; *c*, Santa Catharina meteorite.

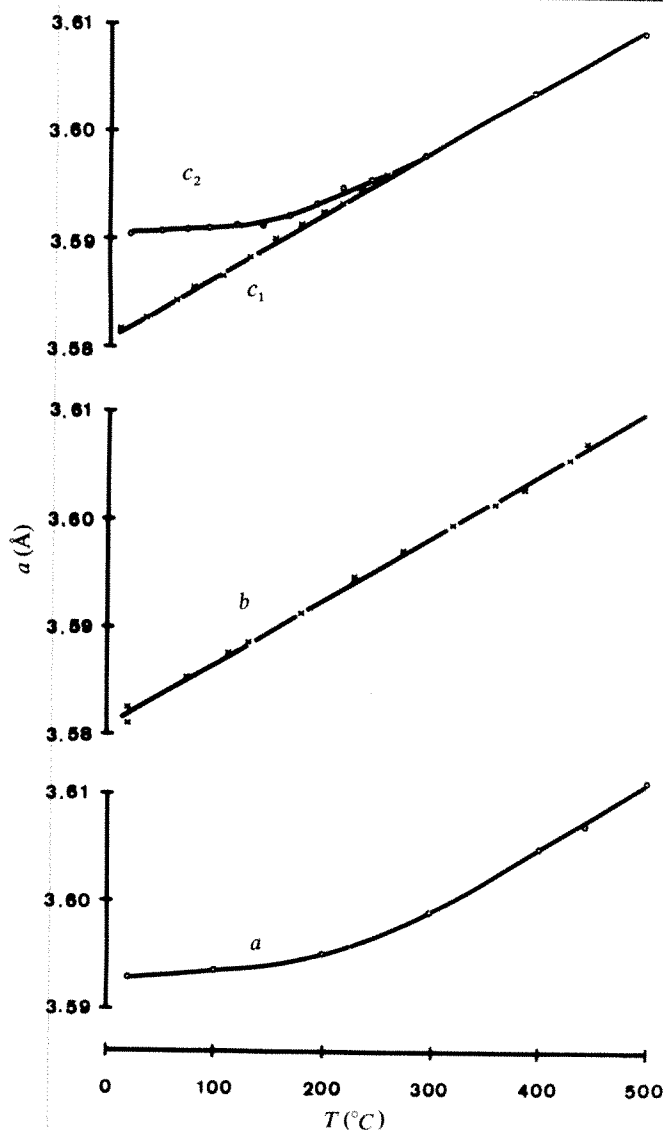


Fig. 3 X-ray lattice parameter variation versus temperature of: *a*, Fe-Ni Invar alloy before irradiation; *b*, Fe-Ni Invar alloy after irradiation; *c*, Santa Catharina meteorite before (c_1) and after (c_2) annealing at 800 °C for 24 h.

ingly low cooling rate which prevails in meteorites when they are inside their parent bodies.

The extensive phase segregation and large proportion of ordered phase observed in the Santa Catharina meteorite are mainly due to its peculiar Ni content (35%), because, as we shall report elsewhere¹⁰, the Invar anomalies which affect both the taenite face centred cubic lattice parameters and the alloy elastic constants also critically affect the phase stability in Fe-Ni alloys.

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These results show that the Fe-Ni phases in Santa Catharina exhibit the same properties of the irradiated Fe-Ni Invar alloy in which phase segregation and radiation-induced ordering occurs with the formation of the L10 superstructure. Basically, the radiation increases the kinetics of the phase segregation and ordering of the alloy. Essentially the same processes occur in the meteorite, but on a much different time scale due to the exceed-

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Ammonia gas concentrations over the Southern Ocean

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Ammonia is the only common, soluble, basic gas in the atmosphere and as such plays an important neutralising, sometimes rate-determining, role in the chemistry of acid gases such as SO_2 , H_2SO_4 and HNO_3 . It also constitutes a reservoir of labile nitrogen in the atmosphere and so must be included in any attempt to describe the cycling of nitrogen through the global environment. We report here values of ammonia concentration measured in Southern Ocean air which are considerably lower than any previously reported¹⁻⁵. For our analyses ammonia was collected on oxalic acid-impregnated filters and determined colorimetrically. In air apparently devoid of contact with land for several thousands of kilometres of travel the mean concentration of ammonia gas was $0.06 \mu\text{g m}^{-3}$ (STP). Concentrations in air apparently affected by the Australian continent were several times higher than in the maritime air.

Measurements were carried out at the Australian Baseline Atmospheric Monitoring Station at Cape Grim (40.7°S , 144.7°E) on the northwestern tip of Tasmania. This is a coastal site at a latitude where only a few per cent of the Earth's perimeter is land and where shipping traffic is minimal. With no land for several thousands of kilometres in directions between 180° and 290° the station is ideally placed to sample the west-southwesterly winds of the roaring forties, which prevail for more than 60% of the time. There are frequent opportunities for sampling Southern Ocean air free from recent contact with land or human activities.

The method used for measuring the ammonia concentration is a simple but sensitive ring oven analytical technique⁶. Samples are collected over a period of 3 (or more) hours on oxalic acid-impregnated filters using a PTFE (Millipore Fluoropore) prefilter heated $\sim 20^\circ\text{C}$ above ambient. This system has been calibrated directly down to concentrations of $1 \mu\text{g m}^{-3}$ and found to be quantitative; particulate collection efficiency on the prefilter has been measured and is 100%. Evolution of ammonia from particles collected on the prefilter was tested by periodically inserting an oxalic acid-impregnated filter upstream of the prefilter. No evidence of significant volatilisation was found. Aitken nuclei were measured with an automated nucleus counter referenced to a faithful replica of the counter developed and calibrated by Pollak⁷.

Experiments were carried out between 1 and 14 August 1979. Conditions remained relatively constant throughout, with gently precipitating bands of cumulus cloud passing over the station at intervals of several hours. Temperature remained between 7.4 and 11.9°C . Wind direction was from the 'baseline sector' (180° to 290°) for 1-4 August and 9-14 August, and from the direction of the Australian mainland (290° - 55°) in the intervening period. Surface pressure charts for these three periods are shown in Fig. 1. Records of temperature, wind direction, Aitken nucleus concentration and ammonia gas concentration are displayed in Fig. 2.

The ammonia and Aitken nucleus concentrations seem to be well correlated, with both following closely the observed changes in wind direction. Figure 2 shows that, after allowing for the scatter in the ring oven results, the lowest values occur for winds from the baseline sector. As Aitken nucleus concentration is a sensitive tracer of continental sources in maritime regions, the most reasonable explanation for these variations is that during the period 5-8 August northerly winds brought continental air from the Australian mainland 250 km to the north, whereas the lower levels of ammonia and Aitken nuclei

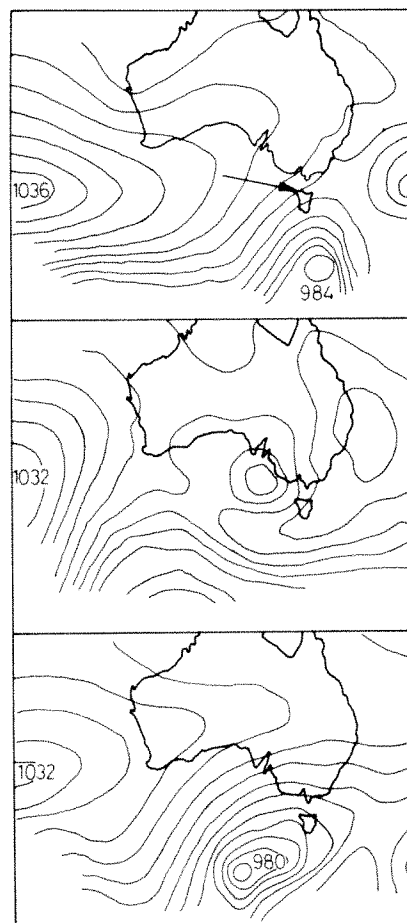


Fig. 1 09.00 EST surface pressure charts. Top, 1 August 1979; middle, 6 August 1979; bottom, 9 August 1979. Isobars are at 4-mbar intervals. The arrow shows Cape Grim.

during 2-4 and 9-14 August correspond with what was a relatively unpolluted southwesterly stream over the Southern Ocean. For 2-4 and 9-14 August we have 41 ammonia gas determinations, giving a mean of $0.06 \mu\text{g m}^{-3}$ (STP) and sample standard deviation of $0.03 \mu\text{g m}^{-3}$ (STP). This is significantly smaller than concentrations reported elsewhere: the range is typically 1 - $10 \mu\text{g m}^{-3}$ (STP) for the troposphere over Hawaii⁵, central Europe^{1,2}, and the Atlantic⁴ and Pacific Oceans³, with lowest values of $0.2 \mu\text{g m}^{-3}$ (STP)^{3,4}. Our values in the non-baseline condition average $0.18 \mu\text{g m}^{-3}$ (STP), with a sample standard deviation of $0.06 \mu\text{g m}^{-3}$ (STP).

Buch⁸ (in 1920) and others^{4,9} have pointed out that the concentration of ammonia near the ocean surface away from stronger sources (such as continents) should be determined by the composition of the surface waters by a Henry's law relationship. The Southern Ocean area is probably one of the few areas where very long air mass trajectories over the ocean enable this hypothesis to be tested. At present we have no knowledge of the distribution of dissolved ammonia in the ocean upwind of Cape Grim to compare with values predicted from our very low atmospheric concentrations. However, it is of interest to investigate whether the atmospheric values are consistent with the range of surface water ammonia concentrations reported from other locations and with those found in coastal water samples taken at Cape Grim.

From Lau and Charlson⁹

$$p\text{NH}_3 = \frac{K_H K_w [\text{NH}_4^+]}{K_b [\text{H}^+]} \quad (1)$$

where K_H is the Henry's law constant for ammonia, K_w the dissociation constant for water and K_b that for aqueous

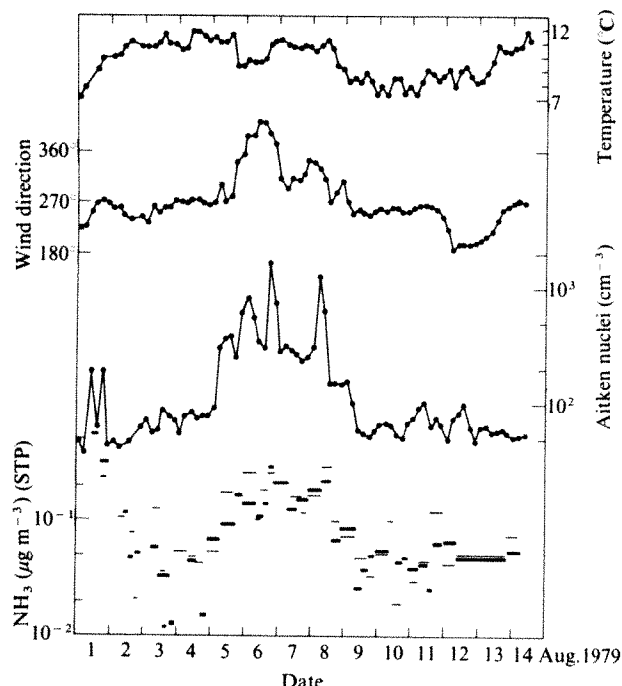


Fig. 2 Records of dry bulb temperature, wind direction, Aitken nucleus concentration and ammonia gas concentration. All points except the ammonia concentrations are 4-hourly averages derived from 24 10-min integrations of the quantity being measured. In the case of ammonia two collectors were used in parallel, with results shown as sets of thick or thin bars. The length of each bar corresponds to the sampling period for that determination.

ammonia. Like Georgii and Gravenhorst⁴, we allow for the fact that the determination of dissolved ammonia does not distinguish between NH_3 and NH_4^+ , by letting

$$a = [\text{NH}_3] + [\text{NH}_4^+] \quad (2)$$

Substitution for $[\text{NH}_3]$ in the expression for K_b gives

$$[\text{NH}_4^+] = \frac{aK_b}{K_b + (K_w/[H^+])} \quad (3)$$

Putting equation (3) in equation (1) and rearranging gives

$$a = \frac{p\text{NH}_3\{K_w + K_b[H^+]\}}{K_H K_w} \quad (4)$$

Values of K_H , K_w and K_b and their temperature dependences are available elsewhere^{10,11}. In the present case we use values for 10 °C, the mean temperature during the experiments: $K_H = 8.26 \times 10^{-3} \text{ atm mol}^{-1} \text{ l}$; $K_w = 2.92 \times 10^{-15} \text{ mol}^2 \text{ l}^{-2}$; $K_b = 1.57 \times 10^{-5} \text{ mol l}^{-1}$.

For the measured ammonia gas concentration of $0.06 \mu\text{g m}^{-3}$ (STP) and coastal water pH of 8.2, equation (4) implies that, if equilibrium is established, the dissolved ammonia concentration is $0.3 \mu\text{mol l}^{-1}$. This falls within the range of dissolved ammonia values found in the literature, <0.05 to ~ 1 or $2 \mu\text{mol l}^{-1}$, with values from the open ocean lying towards the lower end of this range¹². Our own measurements on three separate samples of coastal water collected during the gas measurements yielded dissolved ammonia concentrations of 0.23, 0.30 and $0.55 \mu\text{mol l}^{-1}$. These results must be qualified, as the analyses were performed two weeks after collection; although the samples were stored untreated in polythene bottles at 0 °C and the pH was unchanged over this period it is well-known that storage can affect seawater samples for ammonia analysis¹³.

We therefore conclude that: (1) in winter levels of atmospheric ammonia in Southern Ocean air are 10–100 times less than those reported elsewhere; (2) there are indications that the

mean value observed, $0.06 \mu\text{g m}^{-3}$ (STP), results from a Henry's law equilibrium between atmospheric ammonia and that dissolved in the surface of the ocean.

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Are warm-core eddies unproductive?

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Warm-core eddies¹ are large lenses of subtropical water (radius 100–150 km) wandering through a cooler sea. Figure 1 shows the path of one such eddy (eddy F) in the south-west Tasman Sea. Warm-core eddies in this area are shed by the East Australian Current (EAC)³. Because the EAC is fed mainly by central waters from the western South Pacific^{4,5}, it is low in surface nutrient and generally unproductive^{6,7}. The eddies which it sheds might, therefore, be expected to be equally unproductive. However, the phytoplankton concentration in eddy F increased about fivefold between September⁸ and November 1978⁷. This 'anomaly' is considered here.

Warm-core eddies in the south-west Tasman Sea are recognised by their isothermal layer which extends at the eddy centre

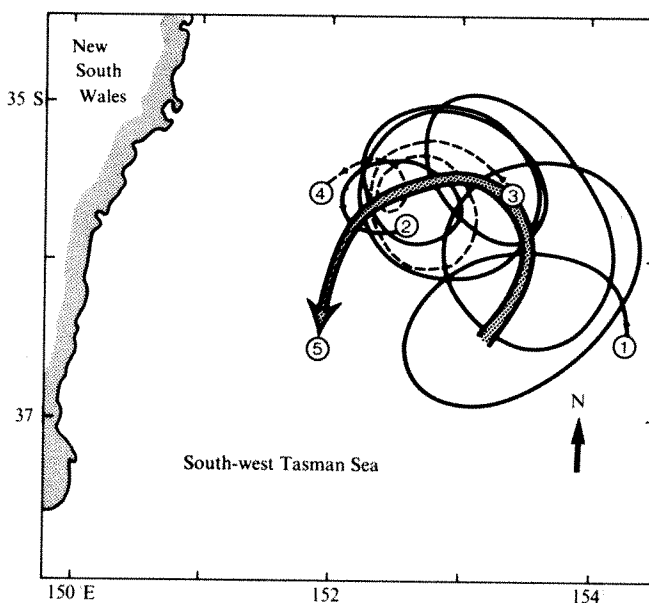


Fig. 1 Probable track of eddy F as deduced from XBT profiles and daily fixes on satellite-tracked buoys drogued at 20 m (ref. 2) (NASA MET SAT NIMBUS-6). Buoy no. 1054 entered the eddy at ① on 16 September and made six complete anticlockwise circuits within the eddy (solid lines) before a systems interruption on 19 October ②. Buoy no. 1132 was released at ③ on 10 October and made two circuits (dashed line) by 19 October ④. The eddy itself seemed to have followed an anticlockwise arc of half a revolution between mid-September and mid-November ⑤.

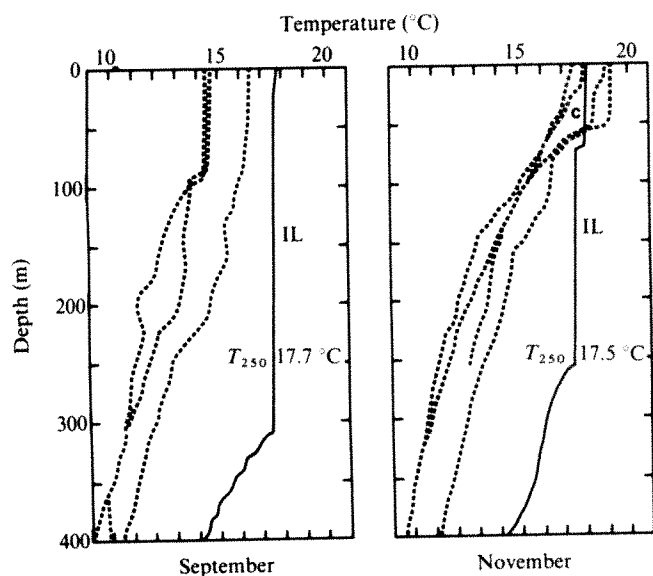


Fig. 2 Temperature profiles taken inside (solid lines) and outside the eddy (dotted lines) in September and November 1978. The eddy is characterised by a deep isothermal layer (IL). In November this was overlain by a slightly warmer 'cap' (c).

to depths as great as 300 m (Fig. 2). A convenient way of identifying particular eddies, and tracking them as they move about, is by their temperature at a depth of 250 m (T_{250}). The centre of eddy F had a T_{250} of 17.5–17.7°C. The T_{250} at the perimeter of the eddy was ~15°C. In the following comparison, locations of $T_{250} > 17^\circ\text{C}$ are regarded as lying near the centre of the eddy and those at $T_{250} < 15^\circ\text{C}$ as lying outside the eddy boundary.

In September 1978 (late winter) the phytoplankton concentration was lower inside the eddy than it was outside (Fig. 3). A second eddy further west (eddy E) had a similar pattern. In November (early summer), the situation was reversed; eddy F now seemed to be far more productive than it had been 2 months earlier (Table 1). The added phytoplankton must either have been brought in from outside the eddy or produced *in situ* from the eddy's own nutrient reserves.

This is undoubtedly the same eddy that was previously under observation in September. Its unique signature is the 17.5–17.7°C isothermal layer (Fig. 2), and its trajectory had been tracked continuously by 'satellite-buoys' drogued at 20 m: buoys drogued at this depth are relatively unaffected by prevailing winds². The most obvious physical change that had taken

Table 1 Comparison of waters inside and outside eddy F during September and November 1978

Month (1978)	T_{250} (°C)	T_0 (°C)	Parameter DML (m)	NO_3 ($\mu\text{mol N l}^{-1}$)	F_M (TU)
September					
Inside	>17	17.7	215–320	2.9–3.2	100–200
Outside	<15	15.3–20.2	37–88	1.1–3.3	200
November					
Inside	>17	18.0–18.2	60–65	0.3–0.8	200–600
Outside	<15	17.5–18.2	60	0.6	200–400

Depth of the mixed layer (DML), T_{250} and T_0 were taken from XBT records. Nitrate was determined by the strychnidine method⁹ using Nansen bottle samples; the values shown are averages for the mixed layer. F_M (*in vivo* chlorophyll *a* fluorescence maximised with 'Diuron'^{10,11}) was measured in a flow-through system with injected dose to give 6 μM concentrations. The source water was pumped through the ship's hull from ~2 m depth. F_M is given in standardised *in vivo* chlorophyll *a* fluorescence units¹² (TU) and is taken as an index of phytoplankton biomass.

place in the interim was the development of a warmer 'cap' (18.0–18.2°C) extending to a depth of 60–65 m (Table 1). The depth of mixing had changed from >300 m (the depth of the deep isothermal layer) to <100 m (the depth of the surface cap). Did this cap develop *in situ* by surface warming, or was it derived from 'outside' surface water which had washed across the top of the eddy?

We first consider the possibility of advection. The EAC tends to advance southwards in the summer, sometimes 'recapturing' eddies shed the previous winter, particularly those located north of Sydney (C. J. Nilsson and G.R.C., unpublished data). However, in November 1978, the EAC was located 640 km north of eddy F. Had warm water from another source washed across the top of eddy F we would have expected the buoys adrift within the eddy (Fig. 1) to be ejected: they were not. Phytoplankton concentrations immediately north of eddy F were much too low to represent a likely source of advected water (Fig. 3). Only beyond its southern limits were November phytoplankton concentrations of the same order as those within the eddy, and here the water was too cold to be a likely source.

For these reasons, we have concluded that the development of the summer cap in eddy F, and the associated increase in phytoplankton concentration, were processes that took place *in situ*, within the confines of the eddy. How did they take place?

Data presented in Table 1 provide a contrast between the conditions prevailing in September and November, both for eddy F and the surrounding waters. The focus is on winter mixing and thermocline formation. In the eddy, winter mixing

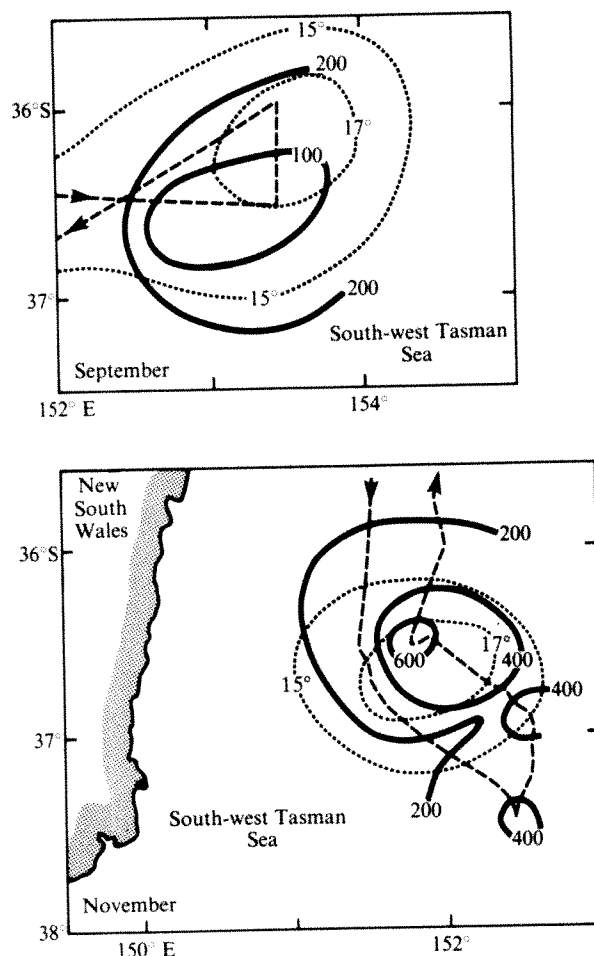


Fig. 3 'Surface' phytoplankton concentration in the region of eddy F in September and November 1978. The perimeter of the eddy is indicated by the 15°C isotherm (at 250 m), the centre by the 17°C isotherm. The phytoplankton contours are *in vivo* chlorophyll *a* fluorescence (F_M) measured underway along the cruise track (for details see Table 1).

had extended deeper, distributing inorganic nitrate fairly uniformly throughout the isothermal layer (Fig. 4). In September, deep-lying stores of NO_3 were being mixed into the euphotic zone of the eddy. By November, most of that which had been introduced in this way was now present in the form of phytoplankton biomass. The mean NO_3 concentration in the upper mixed layer of the eddy (60–65 m) had decreased from 2.9–3.2 $\mu\text{mol N l}^{-1}$ in September, to 0.3–0.8 $\mu\text{mol N l}^{-1}$ in November. The September and November status of eddy F are described in detail elsewhere^{7,8}, as are the conditions in October and December^{13,14}.

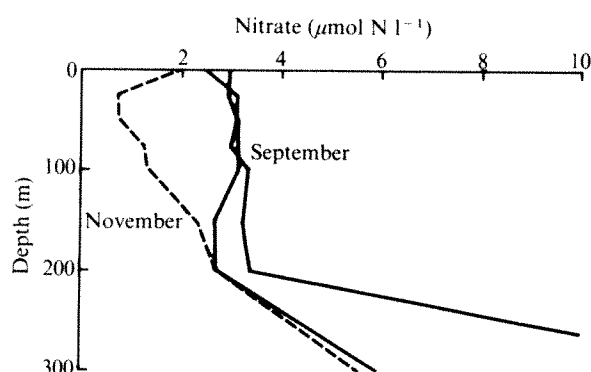


Fig. 4 Nitrate (NO_3) profiles for stations inside the eddy in September and November. September profiles in the eddy displayed nitrate concentrations of the order of 3 $\mu\text{mol N l}^{-1}$ to 200 m. The November profile indicated that more than half of this had been consumed.

These phenomena are explained by Sverdrup's model¹⁵ of environmental control of the spring bloom¹⁶. The Sverdrup model explains the onset of a bloom in relation to the depth of the mixed layer and to the 'critical depth', that is, where respiration losses for the water column equal gains by photosynthesis. In the south-west Tasman Sea, the 1% light level is at a depth of about 75 m (ref. 17), much shallower than the depth to which eddy F mixed in September (250–350 m). Consequently, the standing crop could not increase. Nutrients were distributed throughout the mixed layer, but remained unused for plant production during this light-limited mixing era. When in contact with a mass of air cooler than that at its origin, the eddy took longer to come to thermal equilibrium than did the surrounding sea. Its surface water continued to cool and sink to the level of the permanent thermocline. This process of convective overturn¹ caused deep mixing which persisted through the spring, by which time surface waters outside the eddy had begun to warm. By November, the eddy mixing cycle had ended and it had developed a summer cap (Fig. 2).

We conclude, therefore, that the production of a warm-core eddy is greater than that of the water mass from which it is derived because prolonged surface cooling and deep convective mixing convey previously unavailable nutrients to the surface. This results from the movement of the eddy into a location where the atmosphere is significantly colder.

When eddies are isolated from the surrounding sea, their production is limited by the concentration of nutrients in the winter isothermal layer. Each subsequent year this would be reduced by annual phytoplankton blooms, much of which material would presumably be lost to the system by detrital fallout. In addition to this organic loss, the eddy would lose heat and eventually come to equilibrium with atmosphere and surrounding sea. We would expect these two factors to combine to reduce progressively the scale of consecutive phytoplankton blooms for as long as the eddy remained a discrete water body.

Our studies^{7,8,13,14} show that warm-core eddies can be useful systems in which to study pelagic processes. They can readily be

identified, marked and tracked through time, and are sometimes so discrete that a pulse of primary production can be followed through the food chain.

This letter has dealt principally with production in the eddy proper. Unpublished information indicates that plankton are concentrated at the frontal boundary of the eddy. The factors controlling these concentrations are being studied.

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A revised age for the Donegal granites

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The Donegal granites in north-west Ireland are among the best mapped granitic intrusions in the world¹. They form part of a broad suite of granites intruded during the Palaeozoic but within a relatively small area encompass a wide range of emplacement features. A published Rb–Sr composite whole rock isochron for these granites defined a date of 487 ± 5 Myr (refs 2, 3) whereas K–Ar ages exhibited a spread from 365 ± 8 to 412 ± 8 Myr (refs 1, 4). (All age data are normalized to recently recommended decay constants⁵). Although some doubt was cast on the older Rb–Sr date by Pitcher and Berger (ref. 1, p. 90) and Leake⁶ it has generally been accepted as a more valid estimate of the emplacement age^{2,3,7–9}. Here we summarize the results of Rb–Sr and U–Pb isotopic analyses recently carried out which indicate that the Rosses, Ardara, Trawenagh Bay and Main Donegal intrusions were, in fact, all emplaced close to 400 Myr ago.

Rb–Sr isotopic analyses have been made on more than 70 whole rocks (5–10 kg size) and mineral separates and U–Pb isotopic analyses on zircons and a monazite using methods outlined elsewhere¹⁰. The complete results together with their petrogenetic implications will be presented elsewhere. Here we present a summary of the regression results¹¹ obtained on Rb–Sr age data for those portions of each of four of the main intrusions which best define perfect isochrons. The sum of the squares of the residuals (SUMS) indicates the goodness of fit of the data relative to that expected from the analytical uncertainties. For the Ardara, Rosses and Trawenagh Bay intrusions the best fitting data come from the central portions of the intrusions and the aplites which exhibit a wider spread of Rb/Sr ratio. The outer portions of these intrusions (for which results are not

shown) apparently have variable initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios (compare with refs 12–14). Samples from the Main Donegal Granite had a restricted range of Rb/Sr and the results obtained from mineral separates and the corresponding whole rock samples are given.

The Rosses Complex whole rock data yield a well defined isochron age of 404 ± 3 Myr which is effectively identical to the age of 405 ± 3 Myr obtained for Trawenagh Bay intrusion (Fig. 1, Table 1). The Ardara data scatter significantly at the 5% level and the best fitting age of 405 Myr should be regarded as an approximation to the age of intrusion. Use of York's¹¹ "scatter error" would give a 2σ uncertainty of ± 15 Myr. The five mineral separates (biotite, muscovite, K-feldspar, plagioclase and apatite) and whole rock data points for sample D/12/4 of the Main Granite define an isochron date of 388 ± 3 Myr. This probably represents the time when the intrusion cooled. A second sample (D/17/1) gives similar results for muscovite–biotite–apatite–whole rock, but K-feldspar and plagioclase scatter slightly away from the other points.

A U–Pb isotopic analysis of monazite from the Trawenagh Bay pluton yielded concordant $^{238}\text{U}/^{206}\text{Pb}$, $^{235}\text{U}/^{207}\text{Pb}$ and $^{207}\text{Pb}/^{206}\text{Pb}$ ages whose mean is 411 ± 2 Myr (2σ). U–Pb data for zircons, however, are all discordant with marked inherited radiogenic Pb components analogous to many Caledonian granites from Scotland, north of the Highland Boundary Fault¹⁵. These data strongly suggest melting or assimilation of Precambrian crustal materials or their sedimentary or magmatic derivatives in the genesis of the granites. In this respect it is of interest that the initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratios of the magmas were low (Table 1) which further restricts the sources to having low Rb/Sr ratios. Hence the Donegal granites were generated from low Rb/Sr, low $^{87}\text{Sr}/^{86}\text{Sr}$ crust, possibly in combination with mantle or basaltic lower crustal materials (compare with ref. 10 and ref. 1, Ch. 16).

Pitcher and Berger (ref. 1, p. 90) outlined the probable chronology of these Donegal granites in the order Ardara, Rosses, Main Granite and Trawenagh Bay. The best age data here have been obtained on the Rosses and Trawenagh Bay intrusions which yield similar Rb–Sr whole rock dates indicating that it is exceedingly unlikely that the former preceded the latter by more than 5 Myr. The slight difference between the U–Pb monazite date and the Rb–Sr whole rock date for Trawenagh Bay could merely reflect the uncertainty of absolute calibration of each dating system. The minerals-whole rock Rb–Sr age of 388 ± 3 Myr obtained from the Main Donegal Granite places a minimum on the emplacement age of this intrusion. The highly foliated character of this granite makes it clear that mineral dates should be interpreted with caution. The results from

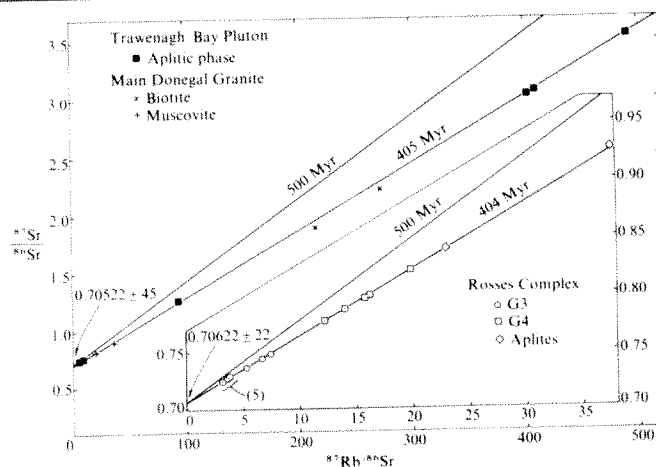


Fig. 1 Rb–Sr isochron diagram for Trawenagh Bay (aplitic phase) and Rosses (G3, G4 and aplites) whole rock samples, and biotites and muscovites from the Main Donegal granite. Reference isochrons are shown.

Ardara indicate that this intrusion was emplaced at about the same time as the others.

The results presented here are inconsistent with previous Rb–Sr age studies^{2,3} in which the Rb–Sr data were interpreted as indicating an age of 487 ± 5 Myr for the Main Donegal, Trawenagh Bay, Thor and Rosses granites, and also inconsistent with a time lapse of ~ 40 Myr between intrusion of the Rosses granite and its aplites². Our results are not inconsistent with those of Long¹⁶ but do resolve the ages more satisfactorily and indicate that the whole rock Rb–Sr isochron date of 352 ± 16 Myr (decay constant not given) he obtained for the Main Donegal Granite does not reflect the emplacement age.

The results presented here indicate that at least a major portion of the magmatism in Donegal took place close to 400 Myr ago and that these granites are temporally related to the 'newer' and 'last' Granites as defined by Read¹⁷. Several implications follow from this. The major deformation and metamorphism in Donegal could be younger than previously thought³, and there is no need to invoke diachronous deformation between County Mayo and County Donegal on the basis of the radiometric age of the Donegal granites^{7,9}. Similarly the need to fit voluminous but localized intrusions of granite at ~ 490 Myr into plate tectonic models of the Caledonides^{7,8} can be done away with. Like most Caledonian granite magmatism the Donegal granites seem to relate temporally to late- or post-closure processes.

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Table 1 Results of regression analysis on Rb–Sr data for Donegal granites

Samples	n*	Age $\pm 2\sigma$ (Myr) $\dagger\dagger$	$^{87}\text{Sr}/^{86}\text{Sr}$ $\pm 2\sigma$	SUMS
Rosses G3, G4, ap. w.r.	15	404 ± 3	0.7062 ± 2	20.4
Trawenagh Bay ap. phase w.r.	6	405 ± 3	0.7052 ± 4	4.60
Main Granite D/12/4 w.r. & m.s.	6	388 ± 3	0.70654 ± 4	6.76
Main Granite D/17/1 w.r. & m.s.	6	386 ± 4	0.70604 ± 1	271§
Ardara G2b, ap. w.r.	7	405 ± 5	0.70655 ± 6	48.0§

ap, aplitic; w.r., whole rock(s); m.s., mineral separates, uncertainty in $^{87}\text{Rb}/^{86}\text{Sr} = 0.7\%$ (1σ) and in $^{87}\text{Sr}/^{86}\text{Sr}$ averages 0.01% (1σ), NBS987 gives $^{87}\text{Rb}/^{86}\text{Sr} = 24.20 \pm 0.05$ and $^{87}\text{Sr}/^{86}\text{Sr} = 1.2004 \pm 0.0005$.

* Number of points.

$\dagger \lambda$ $^{87}\text{Rb} = 1.42 \times 10^{-11} \text{ yr}^{-1}$.

$\dagger\dagger$ Errors refer to least significant digits and are of York's¹¹ a priori type.

§ Significantly large at 5% level.

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The connectance of real ecosystems

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In the work of Gardner and Ashby¹, and May² it has been shown that ecosystem stability (in the sense of Liapunov stability of equilibria³) imposes nontrivial constraints (summarised below) on ecosystem structure, but little has been done to relate this work to observations. One such study was undertaken by Rejmánek and Stárý⁴, who found in a collection of plant-aphid-parasitoid food webs that connectance C (roughly speaking, the fraction of pairs of species that directly interact) decreases like S^{-1} as species richness S (the total number of species) increases. Here I analyse a sample of community food webs (the webs of ref. 4 are sink webs⁵), and find a somewhat slower decrease of connectance with increasing species richness. This behaviour suggests, within the context of May's theory², that complex ecosystems will tend either to be more fragile or to have weaker interspecific interactions than simpler ones.

Connectance, as it occurs in May's theory, is defined precisely as the fraction of non-zero off-diagonal elements of the community matrix². One can also define⁶ an 'average strength', i , of interspecies interactions. Then, for a certain ensemble of randomly constructed systems, May found that the community matrix will almost certainly be stable if

$$i < (SC)^{-1/2} \quad (1)$$

and it will almost certainly be unstable if

$$i > (SC)^{-1/2} \quad (2)$$

with the transition from stability to instability being very sharp if $S \gg 1$.

A food web, which specifies the feeding relationships in a community, can be used to estimate the connectance of the community matrix as follows. First, if species a feeds on species b then the elements A_{ab} and A_{ba} of the community matrix A will certainly be non-zero. If we know all the feeding relationships in a community, we can determine in this way a set of community matrix elements which are certainly non-zero, whence a lower bound C_1 on the connectance. Second, some species will directly interfere with one another, generally in competition for shared

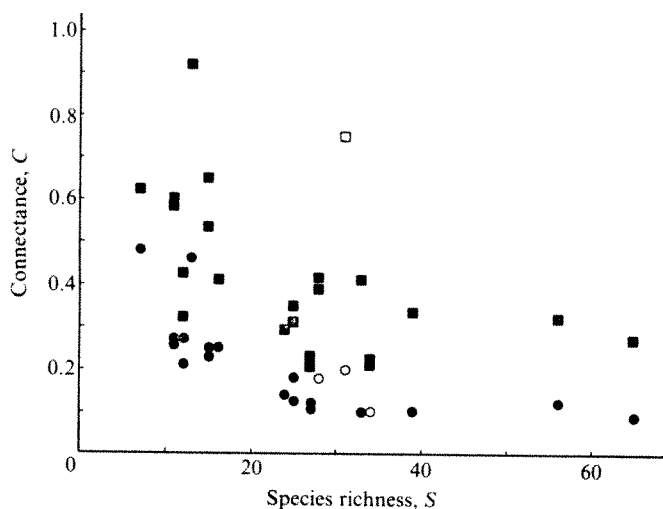


Fig. 1 The estimates C_1 (circles) and C_u (squares), plotted against species richness S for the 24 community food web versions compiled by Cohen⁵. Each open symbol corresponds to two food web versions.

resources. Thus if species a feeds on species b and species c also feeds on species b , then species a and c may engage in interference competition with one another (although they may instead manage to avoid such competition by, for instance, partitioning the food resource in some way). If these two species do interfere with one another, then the elements A_{ac} and A_{ca} will be non-zero. Assuming that any two species which share any food resource interfere with one another, we can determine in this way an additional set of potentially non-zero community matrix elements which, added to the feeding matrix elements previously obtained, yield another estimate C_u of the connectance.

In general there will be additional interactions in a community, due to, for instance, competition for resources other than food, parasitism, and mutualism. If we assume that these interactions are less common than interactions involving food resources, then C_u can be regarded as something like an upper bound on connectance. Otherwise, it can simply be regarded as an estimate which is arrived at in a well-defined way.

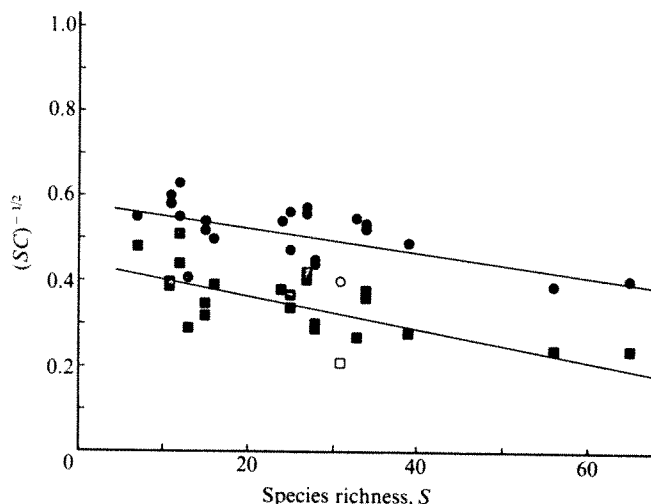


Fig. 2 The estimates $(SC_1)^{-1/2}$ (circles) and $(SC_u)^{-1/2}$ (squares), plotted against species richness S for the 24 community food web versions compiled by Cohen⁵. Each open symbol corresponds to two food web versions. The straight lines are linear regressions of $(SC_1)^{-1/2}$ and $(SC_u)^{-1/2}$ on S .

Cohen⁵ has extracted 24 community food web versions from literature data. While admittedly far from perfect (especially with regard to grouping species into categories), this collection is probably the best statistical base presently available for food web studies. In several cases Cohen gives two (or even three) food web versions for one community; thus one can distinguish for some communities between food web versions of type A, which include only feeding relationships of which the field investigator was certain, and food web versions of type B, which include additional feeding relationships considered probable by the observer. It is not entirely clear whether the most sensible statistical sample is the full collection of all 24 food web versions, the set of 14 type A food web versions, or the set of 14 type B food web versions. Fortunately, the results of this investigation are essentially the same for all three samples.

The connectance estimates C_1 and C_u for Cohen's 24 community food web versions are plotted against species richness S in Fig. 1. There is a clearly discernible tendency for both estimates to decrease as S increases. However, for decreasing connectance alone to account for the stability of complex systems, C must decrease at least as fast as S^{-1} , which is not the case here.

This is made explicit in Fig. 2, which is a plot of the two estimates for $(SC)^{-1/2}$ (hence, according to equation (1), the upper bound on the interaction strength i which allows stability) against S for the 24 Cohen community food web versions. The straight lines are linear regressions on S of the two estimates;

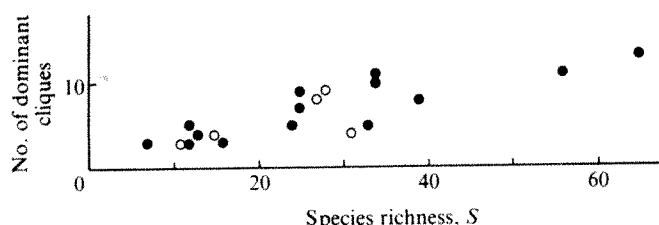


Fig. 3 The number of dominant cliques plotted against species richness S for the 24 community food web versions compiled by Cohen⁵.

both slopes are negative and significantly different from zero (Student's t -test, $P = 0.0036$ for $(SC_1)^{-1/2}$, $P = 0.0006$ for $(SC_u)^{-1/2}$). The significance of this, at least within May's framework², is that unless average interaction strength tends to decrease with increasing S , systems with larger S will be closer to the critical value $i(SC)^{1/2} = 1$ which marks the transition to instability; one expects that such systems would be more fragile in the sense of susceptibility to external perturbations.

Most ecologists would probably expect a decrease in C as S increases, on the basis that ecosystems tend to be organised into

relatively small 'guilds' of species, with most interactions taking place within guilds^{2,6-9}. This idea can be made precise and tested as follows. Define a 'clique' as a set of species with the property that every pair of species in the set has some food resource in common, and define a dominant clique as a clique which is contained in no other clique^{5,10}. These dominant cliques may appropriately be regarded as 'guilds'. In Fig. 3 the number of dominant cliques is plotted against species richness S for the 24 Cohen community web versions. The number of dominant cliques increases with S , although perhaps not as rapidly as one might have expected.

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Effects of leaf age and plant life history patterns on herbivory

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Current theories on plant-herbivore interactions suggest that plant species of different successional status and leaves of various ages differ in their degree of ephemerality and predictability to herbivores, and will therefore exhibit different anti-herbivore characteristics¹⁻⁶. Old leaves and leaves of mature forest plants are expected to be better defended than ephemeral young leaves and leaves of early successional plants. These predicted patterns of plant defence and the resultant patterns of insect grazing are not well documented for natural communities. Field studies have shown that mammalian herbivores in a tropical forest prefer young leaves⁷ and that insect grazing in a temperate forest is heaviest on the young leaves⁸. Laboratory studies have shown that late successional species⁹⁻¹² or plants with certain chemical defences¹³⁻¹⁷ are less palatable for generalist herbivores. Laboratory results depend, however, on the particular herbivore tested, and may not accurately predict rates of herbivory in natural systems. Here I report on rates of herbivory on young and mature leaves from tree species with different life history patterns. Grazing rates (% leaf area eaten per day) on mature leaves of fast growing, shade-intolerant species (pioneers) were an order of magnitude greater than those on slow growing, shade-tolerant species (persistents). Young leaves in both groups of species suffered significantly greater grazing damage than mature leaves.

For this study, tree species were classified into two groups, pioneers or persistents. Persistent species tolerate shade as seedlings and saplings, whereas pioneer species are unable to survive in the shade¹⁸⁻²³. Pioneer species may differ from persistent ones in antiherbivore characteristics because of these differences in their life history patterns. Persistent species grow slowly for long periods of time in the understory before a light gap opens above them, and thus they may be a more predictable resource for herbivores and may be expected to possess costly and effective defences. Mature leaves may be tough and contain

substances that reduce digestibility, such as tannins and resins^{1-4,24,25}. In contrast, saplings of pioneer species may invest in metabolically less expensive toxic chemicals and instead rely primarily on escaping herbivory through fast growth and a patchy occurrence in light gaps¹⁻⁴. Young expanding leaves of both types of plants are a nutritious^{5,26,27} and ephemeral resource, and presumably are not heavily defended by chemicals, especially not substances that reduce digestibility (refs 1-6 but see 7, 28). Synchronous flushing of young leaves by individuals or populations could lead to temporal escape as well as satiation of herbivores^{5,29,30}.

Rates of insect grazing were evaluated for 235 saplings (1-2 m high) of 11 pioneer and 16 persistent species growing in 13 light gaps in the semideciduous forest of Barro Colorado Island, Panama³¹. These 27 species of canopy trees were chosen because both adults and saplings were abundant. I measured total leaf area and area of holes made by herbivores of 1,745 marked young and old leaves at 2-week intervals for three months at the beginning of the rainy season (April-July 1977). Young leaves were measured from the time they emerged from the bud until they were fully expanded and had mature characteristics. Grazing rates were expressed as the percentage of the entire leaf area eaten per day which controls for leaf area in expanding young leaves (see Table 1 for a description of rate measures).

The data on grazing rates (Table 1) suggest that there is a strong relationship of both life history patterns and leaf age with grazing by herbivores. For 23 of the 27 species, there is no overlap between the distributions of pioneer and persistent species in the mean grazing values on mature leaves. There is an order of magnitude difference between the mean grazing rate on mature persistent leaves and that on either mature leaves of pioneers or young leaves of persistents. There is a smaller but significant difference ($P < 0.01$) between the grazing rates on young and mature pioneer leaves. There is no significant difference between the grazing rates on young leaves of pioneer and persistent species.

These results support predictions of current hypotheses¹⁻⁴ which propose that investment in antiherbivore defences will reflect the degree of predictability of each plant or plant part as a resource for herbivores. Low rates of grazing on mature persistent leaves suggest that they are well defended from herbivores, possibly by digestibility reducing substances³. High rates of herbivory on young leaves and pioneer plants suggest that if escape in time or space is operating, it is apparently not so

Table 1 Grazing rates (% leaf area eaten per day) on young and mature leaves of pioneer and persistent species

Persistent species*	Mature leaves	Young leaves
<i>Faramea occidentalis</i>	0.002	0.069
<i>Virola sebifera</i>	0.002	0.108
<i>Prioria copaifera</i>	0.002	0.014
<i>Swartzia simplex</i>	0.003	2.504
<i>Simarouba amara</i>	0.003	0.026
<i>Trichilia cipo</i>	0.003	0.522
<i>Poulsenia armata</i>	0.004	0.027
<i>Desmopsis panamensis</i>	0.004	0.783
<i>Tachigalia versicolor</i>	0.005	0.775
<i>Tetragastris panamensis</i>	0.005	1.477
<i>Protium tenuifolium</i>	0.008	0.928
<i>Hirtella triandra</i>	0.042	0.120
<i>Quararibea asterolepis</i>	0.114	0.316
<i>Alseis blackiana</i>	0.136	0.096
<i>Zanthoxylum panamense</i>	0.136	0.701
<i>Cupania sylvatica</i>	0.311	1.151
Mean†	0.041 ^{abc}	0.631 ^c
Standard error‡	0.024	0.151
No. of species	16	16
No. of plants	120	115
No. of leaves	452	509
Pioneer species	Mature leaves	Young leaves
<i>Didymopanax morototoni</i>	0.007	0.001
<i>Croton bilbergianus</i>	0.025	0.310
<i>Zanthoxylum belizense</i>	0.081	0.624
<i>Spondias radlkoferi</i>	0.186	1.492
<i>Miconia argentea</i>	0.189	0.509
<i>Ochroma pyramidale</i>	0.191	0.178
<i>Alchornea costaricensis</i>	0.210	0.818
<i>Luehea seemannii</i>	0.456	1.108
<i>Cecropia insignis</i>	0.797	0.111
<i>Trema micrantha</i>	1.071	0.053
<i>Cecropia obtusifolia</i>	2.267	1.299
Mean†	0.466 ^{ab}	0.663 ^a
Standard error‡	0.162	0.161
No. of species	11	11
No. of plants	102	105
No. of leaves	386	398

Grazing rates for each leaf were computed as $100 \times (\text{change in hole area}) / ((\text{total leaf area}) \times (\text{no. of days between observations}))$. Significant distortion in the percentage of leaf area eaten does not occur during leaf expansion. Holes of known diameter were punched in 209 young leaves of five pioneer species (*Cecropia insignis*, *Croton bilbergianus*, *Miconia argentea*, *Trema micrantha* and *Zanthoxylum belizense*) and five persistent species (*Desmopsis panamensis*, *Protium tenuifolium*, *Trichilia cipo*, *Virola sebifera* and *Zanthoxylum panamense*). A regression of the percentage change in hole area against the percentage change in total leaf area over time gave a slope not significantly different from 1 ($P < 0.05$, $r^2 = 0.88$ around $\beta = 1$).

*Species are ordered by grazing rates on mature leaves.

†Values followed by the same letter are significantly different, $P < 0.01$ for a and $P < 0.001$ for b and c . Significance levels were determined by a nested 2-way ANOVA considering leaves as replicates on a transformation of the data: $\ln(1,000 \times \text{rate} + 1)$. Although the data were not normally distributed (as a result of many zero values), ANOVA seemed the most appropriate statistical method. The results were not changed when leaves with values of zero damage were excluded. Similar results were obtained when a new rate was calculated by averaging the actual leaf and hole areas per plant rather than the percentage eaten. This second rate of herbivory was computed as: $((\sum_i \text{change in hole area}) \times n_i) / ((\sum_i \text{leaf area}) \times (\sum_i \text{no. of observation days}))$, where i is the i th plant and n the number of leaves. This measure of grazing is less sensitive to the contribution by young leaves that were completely eaten.

‡Standard error of the species means.

effective in reducing losses to herbivores. The magnitude of the differences in grazing rates that I observed emphasises the range of susceptibility to herbivores that exists in natural communities and the presumed variety and effectiveness of different anti-herbivore characteristics.

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Female mimicry in male bluegill sunfish—a genetic polymorphism?

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When male reproductive success depends on male-male competition and aggression, individuals which are at a competitive disadvantage sometimes adopt an entirely different constellation of reproductive behaviours. When such alternative reproductive patterns are practised opportunistically, as when a defeated territorial male adopts a parasitic role, or as part of a developmental sequence, as when younger males are satellites, they can be considered to be part of a single lifetime reproductive strategy. In contrast, when alternatives are available but individuals practise only a single reproductive option throughout their lifetime, the possibility of a genetic polymorphism can be considered. Such genetically mediated alternative reproductive strategies have been hypothesised¹, but the ontogeny of supposed alternative reproductive strategies is seldom known². This letter describes two reproductive patterns in bluegill sunfish (*Lepomis macrochirus*), a nesting male strategy and a female mimic strategy, and demonstrates that an individual male does not practise both reproductive strategies.

Alternative reproductive patterns have been described as 'sneak', 'satellite', or 'parasitic' behaviour, and have been found in a wide variety of taxonomic groups. These alternative patterns are often 'submissive', and can include the display of female characteristics which serve to reduce male aggression³⁻⁵. The most detailed cases of female mimicry as a reproductive option have been reported in scorpionflies⁶, where males increase their frequency of mating by obtaining nuptial gifts from other males; salamanders⁷, where males stimulate rivals to waste their gametes; and fishes, where female mimics attempt to gain access to females attracted to territorial males. In these cases of detailed female mimicry, individuals either were not identified or were observed to practise both typical male behaviour and female mimicry.

For fishes, female mimicry was first described in the 10-spined stickleback, *Pungitius pungitius* L.⁸, and the leaf fish *Polycentrus schomburgkii*⁹, in aquaria. Female mimics have recently been observed in the field in a Michigan population of *Lepomis macrochirus*¹⁰, and in benthic Mediterranean fishes of the genus *Tripterygion*¹¹. The behavioural observations reported here were made while snorkelling or scuba diving in Lake Cazenovia, New York, during the summers of 1976, 1978 and 1979, as part of a long-term study of the mating system of *Lepomis macrochirus*. Following all behavioural observations, individuals were captured with a hand net and stripped of their gonadal products to verify their sex. Because of the difficulty of identifying female mimics in the field, female mimicry in fishes may be much more common than the few reported instances indicate.

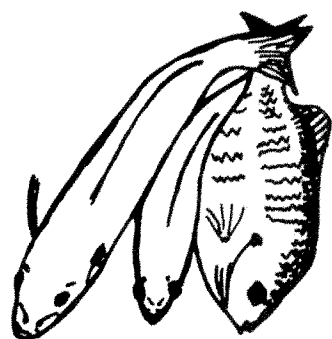


Fig. 1 Nesting male (left), female (right, depositing eggs), and female mimic (small dark fish, between) simultaneously spawning. The line drawing shows the position of the fish—the female mimic is almost lost in the shadow from the larger nesting male.

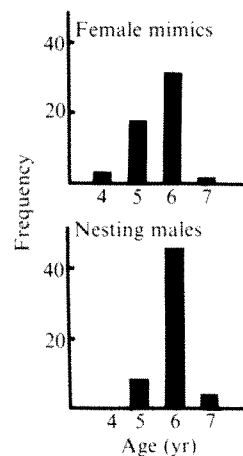


Fig. 2 Age distributions for female mimics (top) and nesting males (bottom).

As is typical of the species, male *Lepomis macrochirus* in Lake Cazenovia occupy nesting territories in densely packed colonies which may contain several hundred nests. Males compete aggressively for position within these colonies, and the ability of a male to maintain residency on a nest is related to its size (unpublished data). This is consistent with aquarium studies of *Lepomis macrochirus* and other sunfishes where body size has been found to be an important determinant of success in agonistic encounters¹²⁻¹⁵. Males which mimicked female behaviour in 1978 (\bar{x} = 126 mm, s.d. = 9 mm, n = 20) were much smaller than nesting males (\bar{x} = 190 mm, s.d. = 13 mm, n = 45), and were never seen to compete aggressively with them. Although the size distribution of female mimics was disjunct from that of nesting males, it did overlap with the lower end of the distribution for spawning females (\bar{x} = 156 mm, s.d. = 19 mm, n = 45).

An actively spawning *Lepomis macrochirus* colony is easily recognised because hundreds of females and males without nests typically aggregate in the water column. To initiate courtship, a female leaves this aggregation and swims towards a nesting male while displaying a coloration pattern (dark background, dark eyes, and pronounced vertical barring) known to reduce aggression in the nesting males of another sunfish, *Lepomis gibbosus*¹⁶. Once the female is in the nest, the male begins to swim in tight circles, turning, with the female following. Every few seconds as the pair turns, the female lies on her side, presses her genital pore against that of the male, quivers, and releases eggs that the male simultaneously fertilises¹⁷.

The behaviour of female mimics is essentially the same as that of functional females. Female mimics do not attempt to construct or defend nest sites, are found in the predominantly female aggregations, and solicit nesting males for courtship. These small males approach the aggressive, territorial males without hesitating or retreating, adopt the dark, barred coloration, and turn in the nest with the resident males. Surprisingly, in some colonies the majority of 'spawning' pairs were nesting male-female mimic pairs. Although this type of 'homosexual' behaviour would not seem to be immediately adaptive, the adaptive significance of female mimicry becomes evident when female mimics spawn as part of a trio (Fig. 1) or larger group which includes a functional female. During these spawning bouts, any eggs fertilised by female mimics are abandoned to the care of the nesting males.

As in the primary males of the bluehead wrasse *Thalassoma bifasciatum*, where sperm competition is also likely to be important¹⁸, the testes of female mimic *Lepomis macrochirus* occupy a greater proportion of their body weight (4.2%) than do the testes of territorial males (1.9%). These large, sperm-laden testes contrast strongly with immature testes or the atrophied testes found in non-breeding individuals. Although released sperm were not visible for either female mimics or nesting males during spawning bouts, a slight pressure to the abdomen of a

female mimic would invariably release copious quantities (compared with a nesting male) of sperm. In the laboratory, these sperm were found to fertilise eggs as readily as those of nesting males. Given that large amounts of active, fertile sperm are present and easily released mechanically, it is likely that during spawning bouts female mimics were releasing sperm and were fertilising eggs. An extremely complex and unusual evolutionary scenario would be required to explain why individuals with exceptionally developed and spermatogenically active testes would adopt an elaborate reproductive pattern, gain access to egg-releasing females, and then fail to release sperm.

Although female mimics are much too small to successfully practise nesting male behaviour during a given breeding season, these small males might continue to grow and eventually reach nesting male size. In such a case, where female mimics represented a developmental stage of nesting males, female mimics should show a younger age distribution. Fortunately, fishes, especially temperate, freshwater fishes offer a distinct advantage over many other vertebrates for studies of the ontogeny of alternative reproductive strategies because they can be accurately aged using the growth characteristics of their otoliths¹⁹. The otoliths of Lake Cazenovia *Lepomis macrochirus* proved to be easily read and unambiguous as to age.

One advantage of using otoliths rather than the more commonly used scales is that with otoliths the annual rings (annuli) can be verified using daily changes in otolith microstructure²⁰. For both female mimics and nesting males, the annuli for the first years of life showed the appropriate number of daily growth increments (E. B. Brothers, personal communication). Although daily increments are more difficult to discern late in life (because of the slowness of growth), the pattern of growth and the appearance of the annuli were unchanged for later years. Daily increments in otolith microstructure have been shown to correspond to daily growth in laboratory reared sunfishes (including *Lepomis macrochirus*), and rings which resembled the annuli of wild caught fish have been induced in laboratory reared *Lepomis cyanellus* by appropriate 'seasonal' temperature manipulations²¹.

Including random population samples, nearly 2,000 fish were aged using otoliths. All otoliths were pooled and read twice, blind with respect to the age, sex and behavioural characteristics of the individual. Using this procedure, female mimics and nesting males were found to show similar age distributions (Fig. 2). Thus female mimics do not represent a normal developmental stage of nesting males. The slightly older age distribution of the nesting males probably results from competition among the males for nesting sites favouring older, larger individuals. Further evidence that female mimics do not mature into nesting males comes from the back-calculated growth rates of the nesting males. If any female mimics were to drastically increase their growth rate and reach nesting male size, the otoliths of some nesting males should show an increase in growth rate late in life. No nesting males showed such a growth increase.

Were alternative behaviour patterns such that individuals could 'switch' from one pattern to another (perhaps better termed 'tactic'), either opportunistically or as a part of development, the genetic substrate for both patterns would be present in every individual. Balancing the relative frequencies of occurrence for these types of alternative patterns is analogous to achieving an optimal mix for the expression of several courtship signals. Each courtship signal need not contribute equally to fitness to be expressed. Demonstrating that female mimics do not exhibit nesting male behaviour, either within a single breeding season or during the course of their ontogeny, however, raises the question of whether this is an example of a genetically maintained, balanced behavioural dimorphism. The next obvious step is to examine the relative contribution of the environment and of the genes to the observed phenotypes.

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At high speeds dolphins save energy by leaping

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An observer may wonder whether a school of 'running' dolphins, consisting of numerous, wildly splashing individuals, is using the most efficient mode of locomotion, because splashing wastes energy. Dolphins exhibit at least three modes of swimming. In leisurely, unhurried motion, they break the surface briefly and gently, often showing little more than the blowhole. At a faster, 'cruising' speed, frequently at 3-3.5 ms⁻¹ (6-7 knots), the animals are seen swimming primarily just beneath the surface, and there is still little splashing. (Behaviour and speeds of dolphin schools were observed from a helicopter and will be described elsewhere by D. A. and W. Perryman.) Swimming speeds in this mode have been measured up to 4.6 ms⁻¹ (9.3 knots). But in the fastest 'running' mode, the animals clear the water in sequential, parabolic leaps, accompanied by considerable splashing on exit and re-entry (Fig. 1). Leaps are interspersed with relatively brief, subsurface swimming. This swimming is common when dolphins are alarmed by vessels approaching within 500 m. We have examined dolphin swimming in terms of energy required per unit distance travelled and report here that beyond a certain 'crossover' speed, leaping must be more efficient than swimming.

The energy cost of leaping must be compared with that of continuous swimming very close to the surface. This is because during rapid swimming, as in escape behaviour, the rate of respiration increases, and the animals are therefore constrained to the surface layer. The extra energy required for jumping is

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approximately (neglecting air resistance)

$$J = WH(1+m) \quad (1)$$

where W is the weight of the dolphin, H is the maximum height of the centre of gravity above the undisturbed sea level, and m is a correction term accounting for the spray carried along with the dolphin as it emerges. The dolphin is approximately neutrally buoyant¹ and so very little energy is required to raise the centre of gravity to surface level. The energy in equation (1) will be compared with that expended by a dolphin swimming continuously with its blowhole just out of the water. This swimming would be roughly equivalent to that of leisurely or cruising swimming, where no more than one-third of the body is out of the water at any instant.

The energy required for swimming at one equivalent body radius beneath the undisturbed surface (that is just submerged) is

$$E = DL \quad (2)$$

where D is the hydrodynamic drag and L is the distance travelled. The drag can be written as²

$$D = \frac{1}{2}\rho_w V^{2/3} C_D \gamma U^2 \quad (3)$$

where ρ_w is the density of water, V is the dolphin volume, C_D the drag coefficient in deep water, γ the correction due to proximity to the surface^{3,4}, and U the swimming speed. Dolphins usually leap only for short periods so that other energy saving aspects of swimming, as when using beat-and-glide or diving⁵, are not applicable.

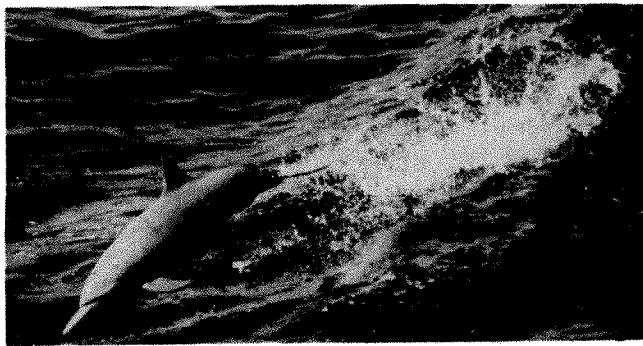


Fig. 1 A 'running' eastern spinner dolphin (*Stenella longirostris*) leaping approximately two body lengths in the rapid swimming mode. Photograph by R. Pitman.

We now write the ratio of energy J/E to find the range of speeds at which this ratio is < 1 , that is leaping is energy sparing. The energies must be compared over the same distance and therefore the length of the jump as a function of swimming speed has to be known. We neglect air resistance. The leaping dolphin has an approximate ballistic trajectory, for which the distance crossed while the centre of gravity of the animal is out of the water is

$$L = \frac{U^2}{g} \sin 2\alpha \quad (4)$$

where g is the gravitational constant and α the emergence angle, measured from the horizon. The maximum height reached by the centre of mass is

$$H = \frac{U^2 \sin^2 \alpha}{2g} \quad (5)$$

The longest leap for given speed U is obtained when $\alpha = 45^\circ$. In this case, designated by subscript 'max',

$$L_{\max} = \frac{U^2}{g}; H_{\max} = \frac{U^2}{4g} \quad (6)$$

Using these results, we obtain the ratio J/E :

$$R = \frac{J}{E} = \frac{\rho_d g V \frac{U^2}{4g} (1+m)}{\frac{1}{2}\rho_w V^{2/3} C_D \gamma U^2 \frac{U^2}{g}} = \frac{\rho_d g (1+m) V^{1/3}}{2\rho_w C_D \gamma U^2} \quad (7)$$

where ρ_d is the density of the dolphin. Equation (7) shows that for a given dolphin swimming as described, the ratio R decreases monotonically as forward speed U increases. Thus, for every species, there will be a range of speeds for which leaping will be more efficient than shallow swimming. The lowest (crossover) speed at which this occurs is obtained by setting $R = 1$ in equation (7). This speed U_c is dependent on the size of the dolphin (through the volume V), its density, its shape (which will define C_D and m), and how far beneath the surface it swims (which defines γ).

Table 1 Predicted values of crossover speed U_c over which leaping becomes energy sparing as a function of body volume

V (m ³)	U_c (m s ⁻¹)	L_c (m)	H_c (m)	E (J)
0.005	3.34	1.14	0.28	17
0.05	4.90	2.45	0.61	368
0.1	5.50	3.09	0.77	928
0.15	5.89	3.54	0.88	1,590
0.2	6.18	3.90	0.97	2,340
0.25	6.41	4.19	1.05	3,160
1	8.08	6.66	1.67	20,100
5	10.57	11.40	2.85	172,000

Predicted leap length and height are shown, as well as the energy required for each leap ($g = 9.8 \text{ m s}^{-2}$). For any given volume, underwater motion at less than U_c is less energy-consuming than leaping the same distance. A volume of 1 m^3 corresponds to a weight of 1,025 kg.

To obtain specific values of the crossover speed, we substitute typical values of the different parameters in equation (7). The dolphin's density is affected by the degree of lung inflation and the extent of fat reserves. We take it to be approximately equal

to the density of sea water¹, that is, $\frac{\rho_d}{\rho_w} = 1$. The spray mass

coefficient is obtained from the longitudinal added mass, defined as the mass of water carried along when a body moves through a fluid. Thus when the dolphin leaves the water, this mass is carried along. The added mass coefficient for fusiform, elongated shapes such as the dolphin's is $^6 m = 0.2$. Hoerner² gives the relationship between the drag coefficient C_D based on volume and skin frictional drag C_f as

$$C_D/C_f = 4(l/d)^{1/3} + 6(d/l)^{1/2} + 24(d/l)^{2/7}$$

which gives a ratio of 8 for a dolphin with an l/d ratio of about 5, where d = diameter and l = length. Hoerner's Fig. 22 shows C_f to be between 0.002 and 0.003 for semi-turbulent flow in the appropriate range of Reynolds number, so that C_D was approximated here as 0.02. The surface effect correction γ is taken as 4.5 when the body is fully submerged, just below the surface. (If the dolphin were an exact body of revolution $\gamma = 5$)^{3,4}. Both C_D and γ should be considered approximations, C_D because it may be a conservative estimate for a flexing body and γ because it is sensitive to submerged depth and decreases rapidly to 1 at a depth three times the body diameter. Substituting all the above values in equation (7) and setting $R = 1$, we obtain

$$U_c = 65.3 V^{1/3}$$

where U_c is in m s^{-1} and V in m^3 . This general equation, while only an approximation for any specific dolphin, should give a good estimate of the expected speeds above which leaping will occur. Table 1 gives the values of the crossover speed, U_c , as a function of body volume.

Table 1 indicates that for the usual size range (0.05–0.10 m³) of most dolphin species^{7,8}, the crossover speed is about 5 m s⁻¹ (10 knots), well within their available range of speeds⁹. During January and February 1977 and 1979, the RV David Starr Jordan approached various dolphin species (*Stenella* spp., *Delphinus delphis*) at speeds of 4.5–5 m s⁻¹ (9–10 knots) in the eastern tropical Pacific. At that speed, the vessel could approach close to all schools as long as they could be tracked. However, it often took an hour for the ship to close onto schools initially seen at about 2 miles (3.6 km) distance. Evidently those dolphins were mostly moving at cruising speeds of 3.5–4 m s⁻¹ (7–8 knots), similar to previous measurements. We observed that the leaping (running) mode occurred primarily after the ship had closed to within 500 m and the animals had become increasingly alarmed and had noticeably increased speed. No measurements of actual speed were possible as the leaping dolphins usually escaped abeam and when followed, continued along curved paths. The speeds attained appeared somewhat greater than that of the ship and were probably in excess of the crossover speed. The actual crossover speed is probably within 10% of the predicted value. Thus, because of their shallow swimming ($\gamma \approx 4.5$), the crossover speed must be approximately 5 m s⁻¹ (10 knots), and dolphins must leap to obtain higher speeds efficiently.

Swimming speed can be estimated indirectly by measuring the distance between the emergence spot and the re-entry splash on photographs similar to Fig. 1, and comparing it to animal length. These distances were within the range of 2–4 m, as expected from Table 1. The height of the leaps (to the centre of gravity) is between 0.6 and 1.0 m, also in agreement with Table 1. Spotted and spinner dolphins (*S. attenuata* and *S. longirostris*) have been observed leaping distances of about three body lengths (5.4 m) when chased closely by speedboats, corresponding to a swimming speed of ~ 7.3 m s⁻¹ (14.6 knots)¹⁰. After escaping purse seine nets, they continued leaping for 1,500 m, performing leaps of ~ 2.3 body lengths, which corresponds to a speed of 6.4 m s⁻¹, also greater than the crossover speed.

Larger cetaceans ($V > 1$ m³, weight > 1 ton) probably cannot leap during fast swimming, as the energy required for leaping increases rapidly with body size (Table 1). Fishes, on the other hand, do not have to break surface to breathe and can stay sufficiently deep for surface proximity effects to be negligible ($\gamma = 1$). Crossover speed would then be increased by a factor of more than 2, and, except for large fish such as istiophorids and some large scombrids, is not likely to be an important factor in swimming.

Although the cetacean running mode has been described in the context of escape behaviour, it could occur in any situation where rapid speeds for short periods were required. Furthermore, leaping behaviour is not restricted to the running mode. At any speed, individual dolphins will often 'splash leap', that is leap out of the water with various contortions so as to re-enter with a large splash. The various attitudes of such leaps indicate, however, that they are not primarily related to horizontal velocity. Their function is most probably that of display-communication, the exact meaning depending on the situation, including the physiological state of the animal.

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Role of the blastocoele microenvironment in early mouse embryo differentiation

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During preimplantation development, the mouse embryo differentiates into an outer layer of cells, the trophectoderm, and an inner group, the inner cell mass. Tarkowski and Wróblewska¹ proposed that this differentiation depends on the position of blastomeres in the morula, with outside cells giving rise to trophectoderm and inside cells producing the inner cell mass. This epigenetic hypothesis has been confirmed by other studies in which blastomere position was altered at the 4-cell or 8-cell stage, thus demonstrating the totipotency of the blastomeres at these stages^{2–4}. It was recently found that the inner cell mass of the early blastocyst is also totipotent and can form trophectoderm when isolated by immunosurgery^{5–8}. Because inner cells apparently become committed during the time that they are exposed to the distinct microenvironment of the blastocyst^{9–11}, we postulated that diffusible components or other factors in the blastocoele may have a role in the commitment of cells in the inner cell mass. We report here results, obtained by injecting donor cells into host blastocysts, showing that totipotent cells exposed only to blastocoele fluid differentiate into morphologically normal blastocysts, whereas those in contact with the blastocyst's inner surface do not.

The influence of the blastocoele microenvironment on development of 8-cell and morula-stage embryos was tested by injecting them into the blastocoele of giant chimaeric blastocysts formed by aggregating 8–10 embryos at the 4- to 8-cell stage. These host chimaeras were cultured 48 h, and at the expanded blastocyst stage they were immobilised with a micropositioner and a hole was made in the host trophectoderm with two hand-held glass needles. The zona pellucida of the donor embryo was either kept intact for exposure to diffusible components of blastocoele fluid or removed to allow intimate contact with the inner surface of the host embryo. The donor embryo was inserted into the blastocoele of the host chimaera, which was then allowed to heal. Donor embryos were scored at 24 and 48 h after surgery for escape, arrest, formation of a blastocoele cavity, or formation of a structure without a blastocoele. Some donor embryos were then removed from the host microenvironment by dissection or immunosurgery¹² and tested for their ability to form trophoblast outgrowths *in vitro*.

Host chimaeras readily accommodated the donor embryo within the blastocoele (Fig. 1a), although the host's cavity began to collapse within minutes after an opening was made in the trophectoderm. The procedure required approximately 5 min and was technically difficult to perform on host embryos that were in early stages of expansion. Ten to fifteen host chimaeras were injected in each experiment. In most chimaeras the trophectoderm healed within several hours, as indicated by the reappearance of the host blastocoele cavity.

Two-thirds of the donor embryos that were injected with their zona pellucida intact at the 8-cell or morula stage developed into morphologically normal blastocysts within the host blastocoele (Fig. 1b, c, Table 1). Approximately a fifth of the donors escaped or were unscorable because the host blastocoele failed to reappear, indicating irreparable damage to the host trophectoderm. A few donors arrested at the 8-cell or morula stage, but

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their frequency did not exceed that of control (uninjected) donor embryos (data not shown). After 48 h of culture some donor embryos hatched but remained free floating within the host blastocoele. When these embryos were removed from the host and cultured, they formed outgrowths that had 15–20 trophoblast giant cells like those found in trophoblast outgrowths from normal blastocysts^{13,14}.

Table 1 Injection of zona-intact 8-cell and morula-stage embryos into chimaeric blastocysts*

Stage of donor	No. of donors injected	Fate of donor		
		Unscorable	Arrested	Blastocyst
8-cell	31	8	2	21
Morula	26	2	6	18
Total	57	10 (18%)	8 (15%)	39 (68%)

* Methods as in Fig. 1.

In contrast to the intact donors, only a tenth of the donors that were injected without a zona pellucida developed into blastocysts (Table 2). More than half the donors, however, formed compact structures that were in intimate contact with host cells and lacked a blastocoele (Fig. 2a). Approximately a third of the donors escaped or were otherwise unscorable owing to collapse of the host trophectoderm; a few donors were arrested at late cleavage stages. The compact structures were generally attached to the inner surface of the host trophectoderm but occasionally were in contact with the host inner cell mass. To determine whether donor embryos formed compact structures as a result of their trophectoderm cells becoming integrated into the host, we labelled donor embryos with ³H-thymidine before transfer to the host blastocoele. Our preliminary experiments show that intense autoradiographic labelling was restricted to the compact structure (Fig. 2a, Table 3). Fewer grains were seen in host trophectoderm cells that were in direct contact with the donor and occasional grains were found in other host cells. We suggest that donor cells were retained within the compact structure and that the host cell labelling may have been due to transfer and reutilisation of thymidine by host trophectoderm. The thymidine labelling conditions used here (10^{-2} μ Ci ml⁻¹ for 4 h) have been shown not to be toxic to early mouse embryo development¹⁵.

Table 2 Injection of zona-free 8-cell and morula-stage embryos into chimaeric blastocysts*

Stage of donor	No. of donors injected	Fate of donor			
		Unscorable	Arrested	Blastocyst	Compact structure
8-cell	54	23	2	5	24
Morula	80	16	3	10	51
Total	134	39 (29%)	5 (4%)	15 (11%)	75 (56%)

* Methods as in Fig. 2.

To define their developmental potential further we removed the compact structures from host chimaeras 2 days after injection and cultured them in conditions that allow post-implantation development¹⁶. In three experiments, 21 of 27 compact structures derived from zona-free donor morulae formed outgrowths. The outgrowths had 5–15 giant cells, and about half had central clumps of cells resembling inner cell mass derivatives (Fig. 2b). Three compact structures failed to develop, two formed blastocysts, and one formed only an inner cell mass derivative.

We interpret the results of injecting zona-intact donors into chimaeric blastocysts to indicate that diffusible substances that are contained within the blastocoele fluid do not inhibit differentiation of totipotent cells into trophectoderm. Austin and Lovelock¹⁷ showed that the rat or hamster zona pellucida is readily permeable to 1,200-molecular weight dyes. Thus, all small ions, amino acids, vitamins, hormones and other small metabolites present in blastocoele fluid should pass through the zona. The mouse zona appears to be permeable to at least some high molecular weight substances, because antibodies can affect development^{18,19} and immunosurgery can be carried out with

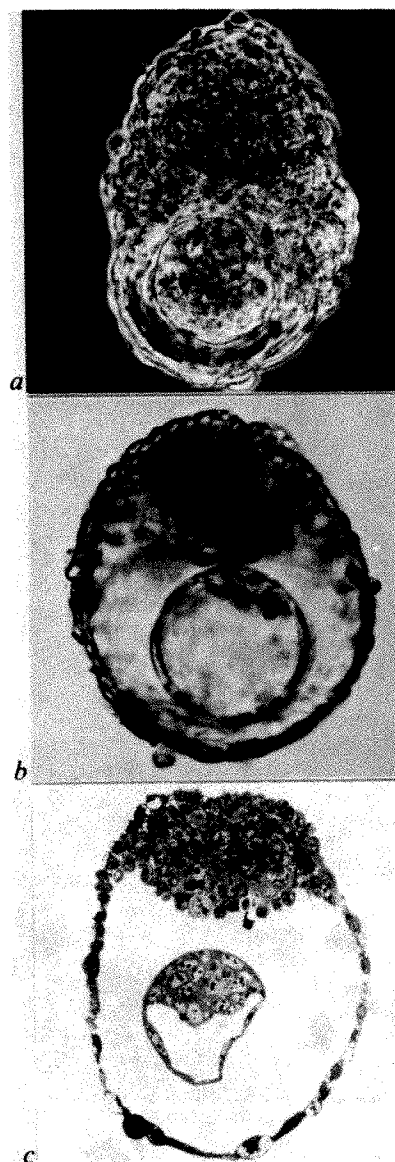


Fig. 1 Chimaeric blastocysts injected with intact donor morulae. *a*, Immediately after injection (phase-contrast, $\times 225$). Donor embryo with intact zona pellucida is in host blastocoele. *b*, After 24-h culture (differential interference contrast, $\times 195$). Donor embryo has developed into morphologically normal blastocyst. *c*, Embryo from *b* fixed and sectioned (bright field, $\times 215$). Embryos were obtained from Dub: (ICR) mice (Flow Laboratories) after superovulation and mating and were cultured in standard egg culture medium as previously described²⁴. Embryos for host chimaeras were obtained on the second day of pregnancy at the 2-cell stage. They were cultured overnight and made into chimaeras by aggregating 8–10 zona-free 4- to 8-cell embryos in phytohaemagglutinin²⁵. Chimaeras were cultured individually in microdrops of medium under a layer of paraffin oil for an additional 3 d. Donor embryos were obtained on the third day of pregnancy at the 8-cell or morula (12- to 16-cell) stage and were inserted into the blastocoele of chimaeras as described in the text. Embryos were fixed and sectioned as previously described¹⁶.

embryos that have an intact zona pellucida¹². As a control, however, we ruptured but did not remove the zonae of eight 8-cell embryos before transfer to host blastocoeles in order to allow free interchange of fluid between the host blastocoele and the donor perivitelline space. Six of the donors developed into blastocysts (one escaped from its zona and formed a compact structure attached to the host trophoblast, and one escaped from the host blastocoele). This result shows that even high molecular weight components of the blastocoele fluid do not alter the differentiation of totipotent donor cells.

The results of injecting zona-free embryos into chimaeric blastocysts cannot be interpreted with certainty because we do not yet know the identity of cells in the compact donor structures. Despite their failure to accumulate blastocoele fluid, the compact structures retained the ability to form trophoblast outgrowths when isolated by immunosurgery. In this respect they resembled inner cells isolated from early blastocysts⁵⁻⁸. Contact with host cells may have altered or delayed the differentiation of donor cells into trophoblast, or it may have physically prevented them from accumulating blastocoele fluid.

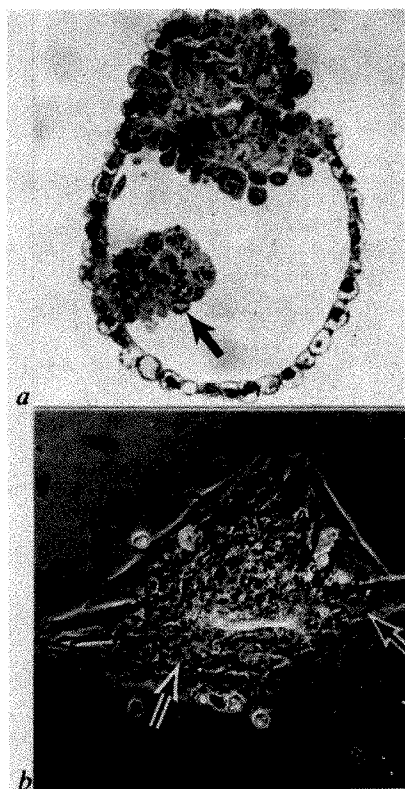


Fig. 2 Structures formed by zona-free morulae injected into chimaeric blastocysts. Host chimaeras were made and donor morulae were injected as described in Fig. 1, except that the zona pellucida was removed by a 3–4-min treatment in 0.5% pronase. *a*, Donor morula labelled with ³H-thymidine, injected into host, fixed after 24 h culture, and sectioned (bright field, $\times 240$). Note grains over nuclei of compact structure attached to inner surface of host trophoblast (arrow). For labelling of donor cells, morulae were incubated for 4 h in $10^{-2} \mu\text{Ci ml}^{-1}$ ³H-thymidine (specific activity, 40 Ci mmol^{-1}), washed extensively, and incubated in unlabelled medium at least 2 h before transfer to the host blastocoele. For autoradiography, embryos were fixed and sectioned as described previously¹⁶, except that the uranyl acetate treatment was omitted. They were exposed to Kodak NTB emulsion for 2 months. *b*, Outgrowth formed by compact structure (phase-contrast, $\times 128$). After injected chimaeras were cultured 48 h in standard medium, compact structures were removed by immunosurgery and cultured in microdrops of modified Eagle's medium on tissue culture dishes (Falcon) for 6 d by the methods previously described¹².

Table 3 Fate of ³H-thymidine label in donor nuclei*

Location of nuclei	No. of nuclear sections scored	Labelled nuclei (%)	Grains per labelled nucleus (mean \pm s.e.m.)
Compact structure	140	94.3	44.5 \pm 31.5
Trophoblast:			
In contact with compact structure	19	89.5	14.3 \pm 11.4
Not in contact with compact structure	286	25.2	2.0 \pm 1.1

* The embryo shown in Fig. 2*a* was fixed and serially sectioned for autoradiography. Grains were counted over nuclei in 15 sections.

Because morphological features are not adequate for distinguishing between these possibilities, we are currently assessing the biochemical status of the compact structures using two-dimensional protein patterns²⁰ to identify inner cell mass and trophoblast. In any case, it seems that intimate contact between totipotent donor embryos and the blastocoele surface of host embryos alters the donors' morphological development. An understanding of the mechanism for this effect may provide clues to the nature of the interaction between inner and outer cells of intact blastocysts. The physical bases of such interactions could include gap junctions²¹, other elements of the cell surface²², or the extracellular matrix²³.

Although we have not excluded the possibility that components of blastocoele fluid can act in concert with non-diffusible components to affect the differentiation of totipotent cells placed in the blastocoele, our results show clearly that blastocoele fluid alone does not alter the early morphological development of totipotent donor embryos. This conclusion is consistent with the fact that trophoblast cells are continuously exposed on their interior surfaces to the fluid that they produce in normal blastocysts. Our results further indicate that the mouse trophoblast can accumulate fluid even when exposed to the higher concentration of ions present in blastocoele fluid, which is consistent with active ion transport¹¹.

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A new immunodeficiency disorder in humans involving NK cells

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Immunodeficiency disorders have provided much information on the development and interaction of the various B and T lymphoid components in the immune system of man. As the lymphoid system becomes increasingly divided into functional subsets of cells it will be important to find immunodeficiencies affecting newly discovered cell types. Natural killer (NK) cells are a recently described but ill-defined subpopulation of lymphocytes which is thought to play an important part in surveillance against tumour development¹. Mice homozygous for the *beige* gene were found to have a selective deficiency in NK function² and were more susceptible to transplantation of syngeneic tumours as predicted^{3,26}. We report here that patients carrying the analogous, autosomal recessive Chediak-Higashi (CH) gene have a profound defect in their ability to spontaneously lyse various tumour cells *in vitro* by either antibody-dependent or independent mechanisms. Since other cell-mediated cytolytic functions were relatively normal, these results suggest that the *beige* or Chediak-Higashi gene in both man and mouse controls NK function.

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The Chediak-Higashi syndrome in man is a rare, genetically determined disease manifested clinically by abnormal leukocyte granulation, defective pigmentation and an increased susceptibility to infections⁴. Humoral immunity and delayed type hypersensitivity are normal⁵ and children usually die of pyogenic infection, presumably resulting from a host defence abnormality related to defective granules in their polymorphonuclear (PMN) leukocytes^{4,6}. Survivors generally succumb to a lymphoproliferative disorder which may be malignant⁷.

In the present study, two male patients, Lewis R. (age 29 yr) and Larry R. (age 28), siblings from a consanguineous marriage, and eight different age and sex matched normal controls were studied in parallel experiments on three separate occasions over a 1-month period. The detailed clinical history and many previous experiments on these patients have been described elsewhere⁸⁻¹⁰. In the two CH patients the immune status was normal with respect to (1) serum immunoglobulin and complement levels, (2) *in vivo* antibody response to immunisation with *Salmonella typhosa* endotoxin and common vaccines, (3) delayed type hypersensitivity to mumps, *Candida*, dinitrochlorobenzene and SKSD (streptokinase-streptodornase-varidase), and (4) *in vitro* proliferative responses to various lectins such as concanavalin A, phytohaemagglutinin and pokeweed mitogen. Phagocytic function was also normal. At the time of study the patients were free of overt infection, and were not undergoing treatment.

To investigate NK function, peripheral blood was separated by standard Ficoll-Hypaque gradient centrifugation and the mononuclear cell band was selectively depleted of most (>90%) monocytes by adherence to microexudates of detached kidney cell monolayers. As shown in a representative experiment (Fig. 1a) lymphocytes from both CH patients were profoundly depressed in their ability to lyse K562 target cells, a human cell line of myeloid leukaemia origin which is the most sensitive NK target described in the human¹¹. Data pooled from three repeat experiments revealed 85 ± 12 lytic units (LU) per 10^6 cells ($n=6$) for age and sex matched normals and <0.1 LU/ 10^6 ($n=2$) for the CH patients when LU were calculated at 20% lysis. The low response in the CH patients was not altered by

Table 1 The range of cytolytic effector functions of peripheral blood cells from donors with Chediak-Higashi syndrome compared with normals

Effector function	Target cell	Normal controls*		CH donors*	
		Max. lysis†	LU/ 10^6 ‡	Max. lysis	LU/ 10^6
(1) NK (lymphocyte)	Alab	40 (100/1)	40 ± 4	3 (100/1)	<0.01
(2) ADCC (lymphocyte)	Daudi	62 (50/1)	164 ± 30	10 (50/1)	7 ± 2
(3) ADCC (monocyte)	HRBC	43 (100/1)	12 ± 2	43 (100/1)	18 ± 5
(4) ADCC (PMN)	HRBC	60 (100/1)	22 ± 6	59 (100/1)	26 ± 4
(5) Spontaneous (monocyte)	TU5	13 (10/1)	ND	12 (10/1)	ND
(6) Spontaneous (PMN)	K562	34 (20/1)	4 ± 1	30 (20/1)	5 ± 1
(7) Lectin induced (lymphocytes)	P815	73 (5/1)	1,000 ± 80	52 (5/1)	550 ± 320
(8) Lectin induced (PMN)	RBL-5	44 (5/1)	100 ± 6	50 (5/1)	80 ± 25
(9) NK after MLC	K562	33 (40/1)	95 ± 35	1 (40/1)	<0.1

Lines 1, 2: Peripheral blood was separated by centrifugation (1,000g) over Ficoll-Hypaque and the mononuclear cell band was depleted of monocytes by adherence (45 min, 37 °C) to fibronectin microexudates of detached TUS kidney cells grown to confluence²². Cell loss was approximately 20% in all donors and contamination of the recovered lymphocytes was <1% monocytes as judged by phagocytosis of latex beads or nonspecific esterase staining and <2% polymorphonuclear leukocytes (PMN) was indicated by Wright's stained cytocentrifuge preparations or staining with a specific monoclonal, anti-human PMN antibody (provided by Dr P. Beverley, University College Hospital, London). Lymphocytes were tested in a 4 h ^{51}Cr release assay against an NK-sensitive target, Alab (breast carcinoma), or an NK-insensitive target, Daudi (IgM bearing EBV lymphoma), pretreated with a 1/50 dilution of rabbit anti-human IgM. Non-antibody treated Daudi cells were lysed 15% by normals and 1% by CH patients at a 50/1 E/T ratio. **Lines 3, 4:** Mononuclear cells (74% lymphocytes, 23% monocytes) obtained by Ficoll-Hypaque separation consisted of approximately 72% E rosette forming cells (T cells), 10% slg^+ cells (B cells) and 27% FcR^+ cells (IgG) with no significant differences between donors. PMN were obtained by dextran sedimentation of Ficoll-Hypaque pellets and consisted of approximately 80% FcR^+ cells, 90% of which phagocytosed bovine erythrocytes coated with rabbit anti-bovine erythrocyte antibody (IgG). Effector cells were tested in an 18-h ^{51}Cr release assay against human erythrocytes (A^+) pre-coated with a 1/10 dilution of rabbit IgG anti-HRBC antibody. **Line 5:** Ficoll-Hypaque purified mononuclear cells were incubated on microexudates and adherent cells (4-7%) were recovered by EDTA treatment and vigorous shaking. Effectors (>90% latex⁺, and nonspecific esterase⁺) were tested in a 48 h, ^3H -thymidine release assay against TU5 target cells (SV40 transformed mouse kidney cells). **Line 6:** Cell pellets from Ficoll-Hypaque were sedimented on dextran followed by re-centrifugation on a Ficoll-Hypaque-Percoll mixture²³. Purified populations were 99% PMN as indicated by morphology and staining with monoclonal anti-PMN antibody. Effectors were tested in a 36 h cytostasis assay as measured by inhibition of ^3H -TdR uptake during a 12-h pulse. Cytostasis against K562 was not inhibited by catalase²³. **Lines 7, 8:** Lymphocytes or PMN prepared as described above were tested in an 18-h ^{51}Cr release assay against P815 (mouse mastocytoma) or RBL-5 (Rauscher leukaemia virus induced murine lymphoma) targets in the presence of 2 or 20 $\mu\text{g}/\text{ml}^{-1}$ phytohaemagglutinin (PHA) respectively. No lysis occurred in the absence of PHA and these concentrations were not toxic. The predetermined optimum PHA concentrations were identical in careful titration experiments, between normals and CH patients. **Line 9:** Mononuclear cells were cultured 7 d together with a pool of irradiated stimulator cells pooled from four normal allogeneic donors as described previously²⁴. Effectors were collected and tested in a 4-h ^{51}Cr release assay against K562 cells, an HLA negative target. Lysis by non-stimulated cultures was approximately half the values shown.

* Two CH patients (Le.R., La.R.) and 2-6 normal, age and sex matched controls were studied in parallel experiments.

† Mean % lysis in triplicate wells at the effector/target ratio given in brackets. These values occurred on the plateau of effector titration curves.

‡ A lytic unit was defined as the number of effector cells required for 20% lysis. ND, not determined.

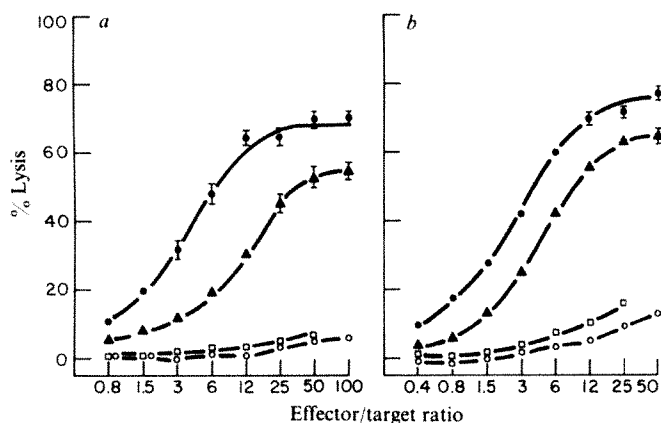


Fig. 1 Impairment of NK and ADCC activity in lymphocytes from Chediak-Higashi (CH) patients. Ficoll-Hypaque separated, peripheral blood lymphocytes were depleted of monocytes on microexudates and incubated with ^{51}Cr -labelled K562 cells, a human myeloid leukemia cell line (a), or antibody-coated Chang cells, a human hepatocarcinoma cell line (b) at various effector-to-target ratios in a 4-h cytolytic ^{51}Cr release assay. ●, J.R.; ▲, J.O. (normal donors); □, La.R.; ○, Le.R. (CH patients). Values represent the mean % specific lysis \pm s.e. in triplicate wells. Lysis of non-antibody coated Chang cells at a 50/1 effector/target ratio was 18, 7, 2, and 1.5% for J.R., J.O., La.R. and Le.R., respectively.

prolonged incubation time (up to 24 h) and was evident in tests of six different NK-sensitive target cell lines including MOLT-4, Alab, CEM, MDA and human fetal fibroblasts (data not shown). The NK defect persisted during a 7-d incubation *in vitro* with or without stimulator cells (Table 1, line 9). As shown in Fig. 1b, lysis of Chang cells, precoated with an optimum dose of rabbit anti-Chang antibody, was also severely depressed. The degree of cytolytic depression was similar against antibody-coated Daudi targets, a human lymphoblastoid cell line (Table 1, line 2) and lysis of antibody-coated RBL-5, a murine tumour, was also markedly impaired although less so than the response against human tumours (unpublished observation). The failure of CH lymphocytes to efficiently lyse human tumours cells was not due to a generalised block in the antibody-dependent cell-mediated cytotoxicity (ADCC) mechanism. Hence mononuclear effector cells and polymorphonuclear leukocytes (PMN) from CH patients lysed antibody-coated human erythrocytes in a normal fashion as shown in Table 1 (lines 3, 4). When combined with the cell fractionation studies shown in Table 2, these results suggest

Table 2 Fractionation of NK and ADCC effectors

Expt	Fractionation	% Lysis of K562*		% ADCC*	
		Normal	CH	Normal	CH
1	FcR ⁺	29	4	36	3
	FcR ⁻	6	0.2	1	0
2	Unseparated	51(14)	5(<1)	59(97)	10(<1)
	G-10 passed	53(53)	1(<1)	64(133)	22(4)
3	Unseparated	22(9)	2(<1)		
	Nylon passed	39(11)	0.4(<1)		
4	Microexudate	60(50)	7(0.3)		
	Microexudate + interferon	75(100)	14(4.0)		

Expt 1: Peripheral blood mononuclear cells were depleted of monocytes by a 1-h incubation on plastic surfaces. Lymphocytes were then incubated 60 min at room temperature in plastic flasks (Falcon) pre-coated with trinitrophenylated (TNP) fetal calf serum (FCS) and rabbit anti-TNP antibody²⁵. Non-adherent lymphocytes (FcR⁻) were recovered in the supernatant and adherent cells (FcR⁺) were eluted with EDTA and scraping. FcR⁺ and FcR⁻ fractions were incubated overnight at 37 °C to allow recovery before assay. **Expt 2:** Mononuclear cells were passed through a 10-ml column of Sephadex G-10 equilibrated with medium containing 20% FCS. Cell recovery was 60% of input. **Expt 3:** Mononuclear cells were passed through a 10-ml column packed with scrubbed nylon wool (Fenwall). Cell recovery was 50% of input. **Expt 4:** Mononuclear cells were depleted of monocytes on microexudates and preincubated for 1 h with 10^3 units ml^{-1} human fibroblast interferon (HEM Research) or medium as control, washed and assayed for cytotoxicity.

* Effectors were assayed in a 4-h ^{51}Cr release assay against K562 cells or RBL-5 cells pretreated with rabbit anti-mouse thymocyte serum. Values represent the mean % lysis in triplicate wells at a 50/1 E/T ratio and the numbers in brackets represent lytic units per 10^6 calculated at 20% lysis.

that the defect in ADCC and NK in CH patients is confined to a subpopulation of FcR⁺, non-adherent lymphocytes selectively blocked in their ability to lyse tumour cell targets.

The selectivity of the antibody-independent NK cell defect is shown in Table 1. Lymphocytes were blocked in their ability to spontaneously lyse tumour cell targets whereas cytolysis of tumour cells by monocytes was normal (line 5). Surprisingly, cytotoxicity by PMN from CH patients against K562 cells was also normal (line 6), as was lectin-generated killing by this cell type (line 8). Since lysosomal enzymes and degranulation are abnormal in CH patients (reviewed in refs 4, 6) these results suggest that lysosomal granules are not involved in PMN cytotoxicity or cytolysis against these target cell lines. The population of T killer cells, as measured by lectin-dependent cytolysis was normal in one patient (Le) but low in the other (La, line 7). A more extensive study is required before a definitive statement on the status of cytolytic T cells can be made. However, T-cell mediated immunity in general was previously shown to be normal in these and other patients as judged by skin testing and proliferative responses to T-dependent mitogens⁸⁻¹⁰.

Table 3 Frequency of target binding cells

Donor	Fractionation	% TBC		
		K562	Molt-4	P815
Normal	Nylon wool passed	24 \pm 4*	37 \pm 3	2 \pm 1
CH	Nylon wool passed	25 \pm 2	36 \pm 4	3 \pm 2
Normal	G-10 passed	15 \pm 2	37 \pm 3	ND
CH	G-10 passed	15 \pm 1	43 \pm 5	ND

Ficoll-Hypaque separated peripheral blood lymphocytes were passed over nylon wool or Sephadex G-10 columns to remove adherent cells and mixed with a fivefold excess of tumour cells. Cell mixtures were centrifuged (150g) 5 min, incubated for 5 min at 37 °C and then stored on ice. Pellets were gently resuspended and the number of lymphocytes binding to tumour cells (TBC) was determined in triplicate samples by size discrimination in a haemocytometer as described previously¹³.

* Values represent the mean \pm variance of %TBC values for two individuals. Nylon wool passed or G-10 passed cells from normals yielded 55%, 46% and 1% lysis of K562, Molt-4 and P815 respectively in a 4-h ^{51}Cr release assay, at a 100/1 E/T ratio. Lysis by CH cells was below 5%. ND, not determined.

Further experiments suggested that the CH defect may lie within the lytic pathway rather than at the level of population size. As shown in Table 3, non-adherent lymphocytes from CH patients were completely normal in their ability to bind and lyse K562 and Molt-4 targets, whereas P815, an NK-insensitive target, was not bound or lysed by CH or normal donors. Most of the lymphocytes binding to tumour cells (TBC) detected in this system have been shown to represent NK cells in the mouse¹² and in the human TBC can be specifically inhibited by pre-incubation of effector cells with solubilised glycoproteins from NK-sensitive targets¹³. Therefore, if the relative number of TBC in a heterogeneous human lymphocyte population is a reliable estimate of the frequency of NK cells, as in the mouse, then these results suggest that NK cells are present in CH patients but do not function.

Suppressor cells in the NK system appear primarily to be adherent, macrophage-like cells (reviewed in ref. 14). Suppressor cells did not appear to be responsible for the low NK response of CH patients as removal of FcR⁻ cells or cells adherent to Sephadex G-10, nylon, wool or microexudates did not restore the low NK response in CH patients (Table 2). In addition no suppression of cytotoxicity was seen in mixtures of CH and normal lymphocytes, even at the highest ratios tested (2/1, data not shown).

The precise location of the defective gene product in the cytolytic pathway is not known but may involve an impairment of cyclic GMP-mediated triggering of NK cells as: (1) cyclic GMP causes a small but significant enhancement of NK activity in mice whereas cyclic AMP decreases the level of cytotoxicity¹⁵; (2) cyclic GMP or inducers thereof have been shown to improve various defects in CH patients such as abnormal granule

morphology¹⁶, chemotaxis¹⁷ and NK function (P. Katz, unpublished observation), and (3) interferon, which boosts NK activity in CH patients (Table 2) is known to cause a transient increase in cyclic GMP levels in lymphoid cells¹⁸.

Thus we have described the first immunodeficiency disorder in humans with an apparently selective deficit in effector functions mediated by NK cells and cells involved in ADCC. Other studies of the more familiar immunodeficiencies, involving impairments of both B and T cells, failed to reveal significant defects in NK function¹⁹ except in the case of severe combined immunodeficiency²⁰. Impairments of ADCC are more widespread (reviewed in ref. 19) and in one study of X-linked agammaglobulinaemia, ADCC was defective whereas NK activity was normal²⁰. In view of the complexity involved it is not surprising that different genes should control unique steps in an otherwise common cytolytic pathway in a single cell, possibly mediating both ADCC and NK activity as previously suggested (reviewed in ref. 21).

It is intriguing to note that 85% of the 53 CH cases on record entered an accelerated 'lymphoma-like' phase¹⁰. Closer scrutiny of this phenomenon may provide the first direct evidence that NK cells in the human are involved in surveillance against spontaneous tumour development.

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Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation

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In near-physiological concentrations, glucocorticoid hormones cause the death of several types of normal and neoplastic lymphoid cell, but the mechanisms involved are unknown^{1,2}. One of the earliest structural changes in the dying cell is widespread chromatin condensation, of the type characteristic of apoptosis, the mode of death frequently observed where cell deletion seems to be 'programmed'^{3,4}. It is shown here that this morphological change is closely associated with excision of nucleosome chains from nuclear chromatin, apparently through activation of an intracellular, but non-lysosomal, endonuclease.

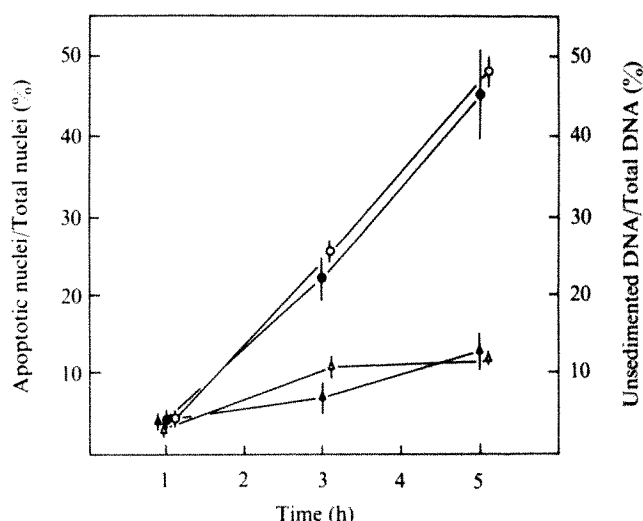


Fig. 1 In methylprednisolone-treated thymocytes the proportion of chromatin cleaved endogenously to small fragments is closely similar to the proportion of nuclei whose morphology shows the generalised chromatin condensation of apoptosis. The graph shows the percentage of total chromatin unsedimented by centrifugation at 27,000g for 20 min in lysates prepared from thymocytes incubated *in vitro* for the times shown, in the presence (●) or absence (Δ) of 10^{-5} M methylprednisolone succinate. Plotted on the same axis is the proportion of nuclei in matched cultures showing the morphology of apoptosis after incubation with (○) or without (△) 10^{-5} M methylprednisolone. Steroid concentrations down to 10^{-7} M gave similar results. All points are means of at least five experiments ± 1 s.e. Cells for chromatin studies were washed free of steroid and growth medium by gentle centrifugation (150g for 10 min) and resuspended in 150 mmol l⁻¹ NaCl, pH 7.0. Aliquots of 600 μ l containing 10^8 cells each were lysed in 10 vol of 25 mmol l⁻¹ acetate buffer, pH 6.6. The lysates were centrifuged at 27,000g for 20 min and acid-precipitable DNA was measured by the diphenylamine reaction¹⁶ in the supernatant, the pellet and aliquots of the original uncentrifuged lysate. Recovery from the 27,000g pellet plus supernatant was consistent at over 82% of the original lysate, and incubation of the lysate at pH 6.6 for up to 20 min at 37°C did not materially alter the proportion of DNA appearing in the 27,000g supernatant. This proportion is expressed as percentage of the total in the uncentrifuged lysate. Cells for morphological study were centrifuged at 150g for 10 min, resuspended in a drop of serum, smeared on glass slides, air dried, fixed in Bouin's fluid and stained by the Feulgen reaction. In each experiment 500 cells were scored for the presence of condensed or normal chromatin.

Suspensions of thymocytes from suckling rats were treated *in vitro* with methylprednisolone succinate for various periods. Thereafter, they were studied by light microscopy to determine the proportion of nuclei with condensed chromatin; matched samples were lysed for measurement of the proportion of chromatin DNA which did not pellet after centrifugation at 27,000g for 20 min. In untreated fresh thymocytes this proportion was near zero, but in treated thymocytes the unsedimented low molecular weight DNA represented an increasing proportion of the total, rising above control levels after 1 h of treatment (Fig. 1). This proportion showed a striking quantitative identity to the proportion of nuclei found to contain condensed chromatin morphologically. The proportion of condensed chromatin also rose progressively with time of exposure to steroid in the treated thymocytes but remained low in the controls (Fig. 1).

Agarose gel electrophoresis showed this low molecular weight DNA to consist of fragments whose molecular weights were integer multiples of a subunit; the length of this subunit—about 180 base pairs—was identical to that of nucleosomes isolated from whole fresh thymocyte nuclei after micrococcal nuclease digestion (Fig. 2). One result of steroid action seemed to be excision from nuclear chromatin of nucleosome chains up to more than 8 units in length. This nucleosome excision was not merely the result of the process of lysis itself, but seemed to have

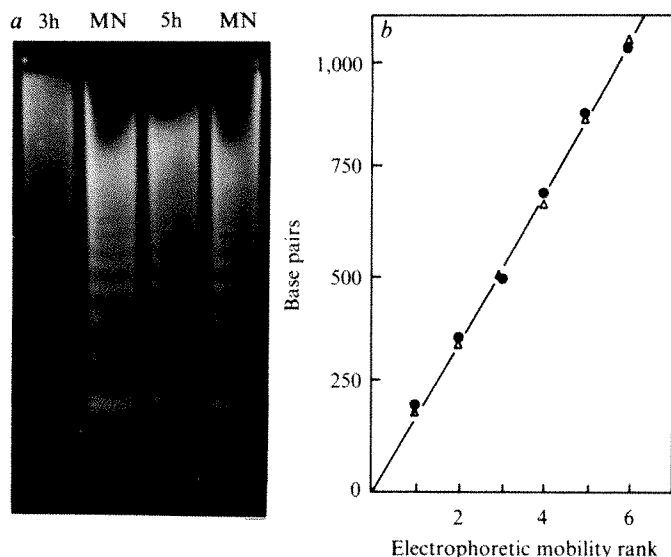


Fig. 2 The low molecular weight chromatin recovered from steroid-treated thymocytes consists of nucleosome chains with a normal repeat length. *a*, Shows a 1.8% agarose gel stained with ethidium bromide and viewed in UV light. The tracks were loaded with DNA from the 27,000g supernatants of lysates of equal numbers of thymocytes after incubation for 3 or 5 h, as indicated, in the presence of 10^{-5} M methylprednisolone succinate, or, for comparison, with a micrococcal nuclease digest of fresh thymocyte nuclei (MN). Thymocyte DNA was concentrated by precipitation in 70% ethanol and 0.13 mol l^{-1} NaCl, dried under vacuum, redissolved in Tris 10 mmol l^{-1} , EDTA 1 mmol l^{-1} and 1% SDS, pH 7.4, and extracted four times through chloroform:isoamyl alcohol (24:1). *b*, Shows the size of the DNA fragments obtained from thymocytes treated for 5 h with methylprednisolone 10^{-5} M (●) and from fresh thymocyte nuclei digested with micrococcal nuclease (△). Mobilities in 1.8% agarose were calibrated against a *Taq* digest of Φ X174.

occurred within intact cells, as untreated cells showed little or no such excision, whereas treated cells showed excision after lysis by several different non-shearing methods, including the use of cation chelators and SDS.

These results show that glucocorticoid induces in thymocytes an endonuclease-like activity whereby well organised chromatin is subjected to multiple double-strand cleavage events, restricted to the inter-nucleosomal DNA. Moreover, the close quantitative identity between the appearance of morphologically condensed chromatin and the products of chromatin digestion implies that this activity may be present only in cells showing the characteristic generalised chromatin condensation of apoptosis. We do not know whether the chromatin excision is itself responsible for the condensation.

The closely delineated size distribution of the excised DNA fragments suggests that the endogenous digestion involves nuclease activity without concurrent protease activity. This is evidence against the view that the endogenous, steroid-associated digestion is merely the result of lysosomal activation. Histochemistry of apoptotic cells also suggests that lysosomal disruption does not occur^{4,5}.

In conclusion, the results demonstrate the presence within glucocorticoid-treated thymocytes of new endonuclease activity which excises well organised nucleosome chains from chromatin. This activity becomes evident after about 1 h of glucocorticoid treatment and correlates quantitatively with the morphologically observed chromatin condensation of apoptosis. Lysosomal enzymes are apparently not involved.

As inter-nucleosomal chromatin breaks can be produced by non-enzymatic means⁶, the evidence that this steroid-associated activity is enzymatic is at present presumptive. However, nucleases capable of mediating this type of chromatin excision are known to be present in the nuclei of thymocytes⁷ and other cells^{8,9}, and inhibitors of macromolecular synthesis prevent chromatin condensation in steroid-treated thymocytes⁴. One

hypothesis, which we are now testing, is that divalent cations mediate the lethal glucocorticoid effects. It is well established in other systems that divalent cations promote chromatin condensation¹⁰, and are required for nuclease activation^{8,9}. Further, certain manifestations of lethal glucocorticoid action on thymocytes have been shown to be cation dependent^{11,12}, although these manifestations were observed many hours later than the chromatin condensation and nuclease activation recorded here. Internucleosomal chromatin breakage has been recorded in lymphocytes and fibroblasts during death induced by immunological attack (although apparently not during lysis as a result of viral infection)¹³, and also in the terminal differentiation of normoblasts¹⁴ and lens cells¹⁵. This raises the question of whether a class of endonuclease exists whose activation is associated with the programmed destruction of the genome.

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Insulin-like stimulation of glucose oxidation in rat adipocytes by vanadyl (IV) ions

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The mechanism of insulin action is still unknown¹. One approach to this problem is to apply substances which mimic the action of the hormone to target cells. Ouabain and deprivation of extracellular K^+ (refs 2, 3), which inhibit the active transport of Na^+ and K^+ ions, are both known to activate glucose transport and oxidation in isolated adipocytes. Vanadate (V) ions have recently been shown to act as very efficient inhibitors of the sodium pump or $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ in *in vitro* preparations⁴. They have a natriuretic and diuretic effect in rats⁵ and a positive inotropic effect on cat heart muscle⁶. Many tissues contain vanadium at a concentration of about 0.1–1.0 μM (ref. 7) and so endogenous vanadate could be a physiological regulator of the sodium pump. But this is still open to debate, because the bulk of the vanadium is probably in the vanadyl (IV) form^{8,9} and VO^{2+} ions bind tightly to proteins^{8,10}. VO^{2+} is a relatively ineffective inhibitor of $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ *in vitro*^{8,11}. We report here that externally applied vanadate ions at low concentrations mimic fully the effect of insulin on glucose oxidation in rat adipocytes. However, this simulation seems to be due mainly to the effects of vanadyl (IV) ions, probably produced within the cells, and not primarily to inhibition of the sodium pump. Also, externally applied vanadyl (IV) ions stimulate glucose oxidation substan-

tially. Vanadyl ions are known to be powerful inhibitors of alkaline phosphatase¹² and we therefore consider the possibility that they inhibit a cellular phosphatase activity. An early event in insulin action may involve alteration of the degree of phosphorylation of protein(s) involved in regulation of sugar transport.

The basic effects of insulin and vanadate on glucose oxidation in adipocytes are summarised in Table 1. There is evidence that in adipocytes the rate of oxidation is limited by the entry of glucose into the cell^{13,14}. Insulin alone routinely stimulated the rate of glucose oxidation by three- to fourfold, and an optimal concentration of vanadate ions in the medium (see Table 1 and Fig. 2) normally produced a 70–100% maximal activation of glucose oxidation. As reported previously¹⁶ externally applied ATP partially inhibited the effect of insulin, and this is also the case with vanadate (Table 1). Also, a non-hydrolysable analogue adenosine 5'-(β , γ -imido)triphosphate (AMP-PNP) was ineffective, as found before with insulin¹⁶. Further evidence for the similarity of the effects of vanadate and insulin is the identical stimulation of glucose oxidation over a wide range of glucose concentrations. The degree of stimulation falls as the glucose concentration in the medium is raised, suggesting that the site of activation is the saturable glucose transport system. Removal of Ca^{2+} from the medium did not affect stimulation of the glucose oxidation by either insulin or vanadate (data not shown).

Table 1 Stimulation of glucose oxidation in fat cells by insulin and vanadate. Effects of ATP and AMP-PNP

Addition	¹⁴ CO ₂ production* (c.p.m. per 2 h \pm s.e.m.)	% Maximal stimulation
None	5,800 \pm 200	0
Insulin (10 ng ml ⁻¹)	24,500 \pm 400	100
Insulin (10 ng ml ⁻¹) + ATP (0.4 mM)	15,000 \pm 100	48
Vanadate† (0.1 mM)	23,000 \pm 220	90
Vanadate (0.1 mM) + ATP (0.2 mM)	18,700 \pm 240	68
Vanadate (0.1 mM) + ATP (1.6 mM)	13,600 \pm 200	41
Vanadate (0.1 mM) + AMP- PNP (0.1 mM)	23,200 \pm 140	92

Isolated fat cells were prepared from Sprague-Dawley rats (80–120 g) according to Rodbell¹⁵. Glucose oxidation was measured by conversion of D-[U-¹⁴C]glucose to ¹⁴CO₂ (ref. 15). The final reaction volume of 0.5 ml contained 0.16 mM glucose and about 3×10^5 fat cells, suspended in Krebs-Ringer buffer (pH 7.4) consisting of: NaCl, 110 mM; NaHCO₃, 25 mM; KCl, 5 mM; KH₂PO₄, 1.2 mM; CaCl₂, 1.3 mM; MgSO₄, 1.3 mM and bovine serum albumin, 7 mg ml⁻¹.

* The absolute rate of glucose oxidation with insulin was 4.8 nmol per 2 h. The assay was run in duplicates.

† Sodium orthovanadate (Na₃VO₄) was applied.

Inhibition of (Na⁺ + K⁺)ATPase and active Na and K transport in red cells¹⁷ and squid axons¹⁸ by vanadate ions has been shown to require the presence of K⁺ in the medium. This was clearly not the case for stimulation of glucose oxidation by externally applied vanadate (see Table 2). Removal of K⁺ from the medium inhibits sodium pump activity and gave a partial stimulation of glucose oxidation as described previously². But addition of vanadate in the K⁺-free medium gave a further near maximal stimulation. In fact the vanadate was somewhat more effective in the K⁺-free than in the normal K⁺-containing medium (compare lines 3 and 6 of each experiment, Table 2).

The data in Fig. 2 provide evidence that the principal form of vanadium involved in stimulation of glucose oxidation is the vanadyl ion. The K_{0.5} for externally applied ortho vanadate was about 20 μ M. Addition to the medium of the reducing agent glutathione (GSH) (see refs 4, 11) lowered the concentration of added vanadate for a half maximal stimulation by almost one order of magnitude (K_{0.5} = 3 μ M). GSH (1.2 mM) itself

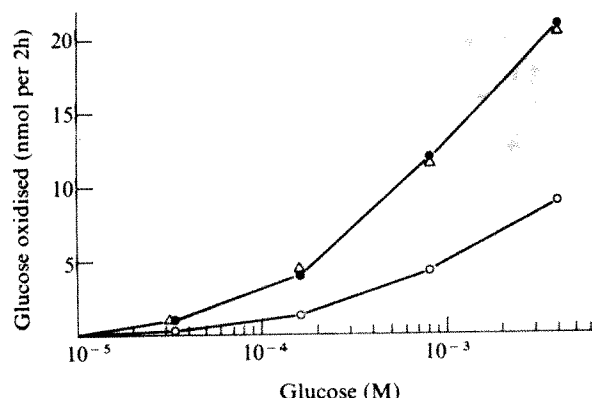


Fig. 1 Rates of glucose oxidation with insulin and vanadate at increasing concentrations of glucose. Isolated fat cells (about 3×10^5 cells) were incubated for 2 h at 37°C in the conditions described in Table 1, except that the D-[U-¹⁴C]glucose was diluted with unlabelled D-glucose to give the final concentrations indicated. Samples containing either insulin (10 ng ml⁻¹, ●), Na₃VO₄ (0.2 mM, △) or no addition (○) were run in duplicate.

produced a small but significant stimulation of the rate of glucose oxidation in these cells (5–15% of the rate of saturating insulin concentrations)¹. Adrenaline (200 μ M) also increased the effectiveness of suboptimal concentrations of vanadate (not shown). Addition of vanadyl ions directly to the incubation medium produced a substantial but incomplete stimulation of glucose oxidation, with an apparent K_{0.5} of ~25 μ M. The presence of GSH did not affect maximal stimulation or the apparent affinity for this effect of VO²⁺. Control experiments showed that solutions of vanadyl sulphate above 100 μ M at neutral pH become cloudy, and so the lack of full stimulation is probably attributable to limited solubility of VOSO₄.

In red cells externally applied vanadate is transported into the cell on the anion carrier¹⁰, and is reduced largely to the vanadyl ion (IV) which itself is bound tightly to haemoglobin⁸. In adipocytes also, preliminary measurements using the vanadyl (IV) ESR signal, indicate that externally added vanadate or vanadyl ions penetrate into the cells and vanadate (V) is reduced to the vanadyl (IV) state by the endogenous reducing compounds (H. Degani, Y.S. and S.K., unpublished data). Both internally generated vanadyl ions and externally applied vanadyl ions become bound to intracellular components (H. Degani, Y.S. and S.K., unpublished). Glutathione is known to be lost from many cells when they are incubated in a GSH-free medium¹⁹. Thus externally applied GSH (see Fig. 2) may enter the adipocytes and supplement the endogenous reducing power, shifting the ratio of vanadate to vanadyl in the direction of vanadyl. The higher concentrations of VO²⁺ required, compared to vanadate plus GSH could reflect slow penetration or binding of VO²⁺ to

Table 2 Stimulation by vanadate ions of glucose oxidation in a K⁺-free medium

Expt	Condition	¹⁴ CO ₂ production (c.p.m. \pm s.e.m.)
26979	K ⁺ -containing medium	7,775 \pm 426
	+ insulin (4 ng ml ⁻¹)	23,200 \pm 201
	+ Vanadate (100 μ M)	14,850 \pm 652
	K ⁺ free medium	17,000 \pm 1500
241079	+ Insulin (4 ng ml ⁻¹)	23,850 \pm 50
	+ Vanadate (100 μ M)	22,875 \pm 727
	K ⁺ containing medium	5,650 \pm 351
	+ Insulin (10 ng ml ⁻¹)	20,100 \pm 500
	+ Vanadate (100 μ M)	14,425 \pm 20
	K ⁺ -free medium	7,700 \pm 201
	+ Insulin (10 ng ml ⁻¹)	19,650 \pm 952
	+ Vanadate (100 μ M)	16,400 \pm 301

Glucose oxidation was carried out as described in the legend to Table 1. In the K⁺-free medium KCl was omitted and NaH₂PO₄ replaced KH₂PO₄.

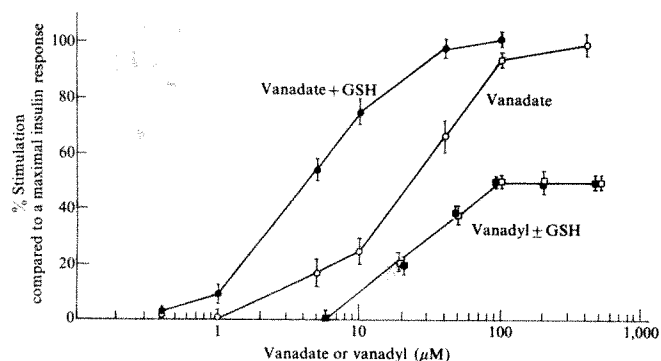


Fig. 2 Rates of glucose oxidation at 0.14 mM glucose were measured as in Table 1. In this experiment maximal stimulation by insulin was 445% of the control value. Where indicated reduced glutathione was present at a concentration of 1.2 mM. The small stimulation of glucose oxidation by GSH alone was subtracted from that observed in the presence of vanadate or vanadyl ions.

the albumen in the medium. Specific ^{125}I -insulin binding to the adipocytes was not affected by vanadate (not shown).

In the light of these results it is unlikely that added vanadate acts primarily by inhibiting $(\text{Na}^+ + \text{K}^+)\text{ATPase}$. The vanadate ion is known to affect several enzymes involved with phosphate or phosphate substrate metabolism²⁰. However, the effective species in our experiments seems to be the vanadyl ion which is known to inhibit strongly alkaline phosphatase¹². If in the adipocytes the vanadyl ions inhibited a phosphatase activity this could imply a connection between the degree of phosphorylation of cellular proteins (the glucose carrier itself?) and regulation of glucose entry by insulin or vanadate. Insulin has been shown to stimulate phosphorylation of certain proteins in adipocytes equilibrated with orthophosphate^{21,22}. The steady state level of phosphorylation should reflect the balance between the relevant phosphorylating and phosphatase activities. Insulin action could be accompanied by inhibition of a phosphatase activity as proposed for added vanadate or vanadyl ions. Alternatively the hormone action may involve stimulation of phosphorylating enzymes. Inhibition of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ by removal of external K^+ or by ouabain or vanadate, should save cellular ATP, making more available for phosphorylation.

Vanadium is found in many cells, including classical target tissues for insulin such as skeletal muscle and liver. If, as seems likely, it is mainly in the vanadyl form and at physiological concentrations of 0.1–1.0 μM , it is available for interaction with cellular phosphatases, it could play an important part in controlling protein phosphorylation *in vivo*. An attractive possibility is that insulin bound to the cell exterior alters either the ratio of vanadyl to vanadate concentrations, or binding of the VO^{2+} ion to cytoplasmic proteins, that is VO^{2+} could be a second messenger. Possible functions of internal vanadyl ions are being investigated.

As far as we are aware this work represents the first demonstration of an effect by vanadyl ions on an important physiological process in whole cells. Whether this finding has wider implications for regulation of cytoplasmic and membrane processes by vanadyl ions remains to be established.

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A site for the potentiation of GABA-mediated responses by benzodiazepines

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The benzodiazepines have been well characterised as minor tranquillizers and attempts to explain their unique spectrum of activity have included suggestions that they may interact with a variety of neurotransmitter systems¹. Recently, a possible interaction with the γ -aminobutyric acid (GABA) system has received most attention. Benzodiazepines potentiate the actions of both synaptically released and exogenously administered GABA^{2–4} on mammalian neuronal preparations but the site of action within the GABA response mechanism has not been determined. Binding studies suggest that benzodiazepines combine with highly specific sites in the neuronal membrane⁵ and that these sites have some indirect association with GABA receptors^{6–11}. To investigate this association further in a functioning GABA system, quantitative studies have been made *in vitro* on neuronal depolarisations mediated by GABA receptor activation. Evidence has already been presented¹² that bicuculline is most probably a competitive antagonist at the GABA receptor while picrotoxin acts as an antagonist at a separate site. Here flurazepam is shown to attenuate preferentially the action of picrotoxin rather than bicuculline and a model is suggested for the site of action of these drugs within the GABA response mechanism.

Slices of rat cuneate nucleus were prepared as described previously¹³ and arranged in a two-compartment bath such that drugs could be superfused over the terminal regions of the afferent fibres to the nucleus and consequent changes in membrane polarisation could be recorded¹². Muscimol (Fluka) was used routinely as the agonist for GABA receptors in these quantitative studies to avoid the complications which arise when agonists such as GABA are rapidly removed by a saturable uptake process¹². Muscimol depolarised the nerve fibres in a dose-dependent manner. In the presence of 10^{-6} M flurazepam-HCl (Roche), the dose-response curve was usually shifted to the left in a parallel fashion (Fig. 1). This potentiation of muscimol was not very large but, in replicate experiments, it proved to be significant with a mean muscimol dose ratio of 0.808. At a higher concentration of flurazepam, 10^{-5} M, there was no further increase in the potentiation of muscimol and at the lower concentration of 10^{-7} M there was no significant potentiation at all.

With flurazepam still present, either (+)bicuculline (Sigma) or picrotoxin (Sigma) were added to the superfusion fluid¹² and the lower part of the muscimol dose-response curve was re-determined. Both antagonists displaced the dose-response curve to the right in a parallel fashion. For each concentration of antagonist, the equipotent muscimol dose ratio was determined and the results are presented in the form of Schild plots in Fig. 2. Control data obtained from similar experiments in the absence

of flurazepam¹² are also shown. As there was no way of determining whether bicuculline and picrotoxin antagonised the potentiating effect of flurazepam as well as antagonising muscimol, the results have been calculated in two ways: (1) assuming no antagonism of the potentiation *per se* and (2) assuming complete antagonism of the potentiation at all concentrations of antagonist. When assumption (1) was made, the potency of bicuculline as an antagonist of muscimol was not significantly reduced by flurazepam 10^{-6} M. The potency of picrotoxin, however, was consistently reduced, 2.67 times as much picrotoxin being required to achieve the same degree of antagonism as in the control experiments. When assumption (2) was made, there was an extra small reduction in the potency of both antagonists but the overall reduction in picrotoxin potency was still considerably greater than the reduction in bicuculline potency. An increase in the concentration of flurazepam to 10^{-5} M caused no further reduction in the potency of picrotoxin and flurazepam 10^{-7} M was ineffective. Other benzodiazepines must be tested to establish whether the observations with flurazepam reflect a general property of this class of drug.

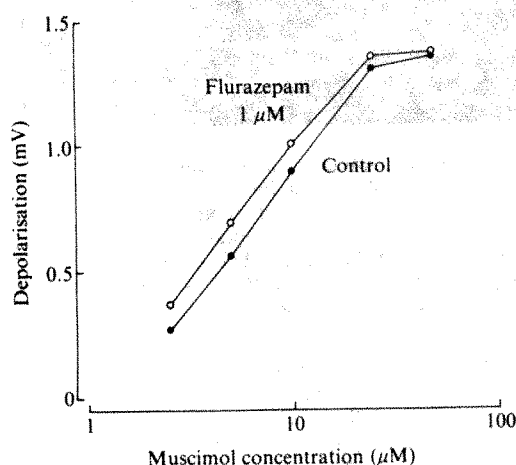


Fig. 1 Depolarisations of afferent nerve fibres in a slice of rat cuneate nucleus by 2 min superfusions of muscimol. Peak responses are plotted against log muscimol concentration before (●) and during (○) the superfusion of flurazepam 10^{-6} M. Each point is a single response. The equipotent muscimol dose ratio in this experiment was 0.771. In replicate experiments, the equipotent muscimol dose ratio (mean \pm s.e.m.) in the presence of flurazepam 10^{-6} M was 0.808 ± 0.047 ($n = 24$) which is significantly less than 1 ($P < 0.01$); in the presence of flurazepam 10^{-5} M it was 0.837 ± 0.086 ($n = 12$) and in flurazepam 10^{-7} M it was 0.905 ± 0.047 ($n = 9$). In this, as in other experiments, flurazepam had little direct effect, the most usual response being a small and transient depolarisation. In control experiments, there was no sign of sensitisation or tachyphylaxis upon repeated administration of muscimol.

Interpretation of these results in terms of the site of action of flurazepam depends largely on a knowledge of the sites of action of the GABA antagonists bicuculline and picrotoxin. In the preparation used here, and in other neuronal preparations from vertebrate species, the evidence points to bicuculline being a competitive antagonist at the GABA receptor while picrotoxin acts at a different site¹². This distinction is corroborated by the demonstration of separate binding sites for bicuculline and picrotoxin^{14,15}. Penicillin appears to act as a GABA antagonist at yet another site which may be the chloride ion channel opened by the GABA receptor¹⁶. A hypothetical model of the GABA response mechanism incorporating these different sites is shown in Fig. 3.

The site of action suggested for flurazepam is based on the observation of a preferential reduction in the potency of picrotoxin rather than bicuculline and an assumption of a single site for benzodiazepine action. It seems unlikely that flurazepam and picrotoxin competed for a common site since increasing the concentration of flurazepam did not result in a further reduction

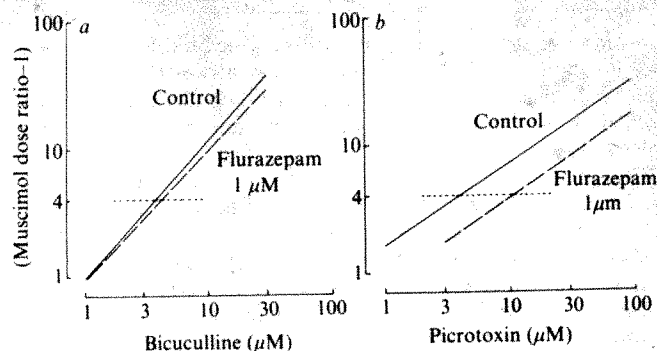


Fig. 2 Schild plots of the antagonism of muscimol by bicuculline (a) and picrotoxin (b). log (muscimol dose ratio - 1) is plotted against log (antagonist concentration) and each line was calculated by least squares regression analysis from 18-28 points. The continuous lines represent antagonist potency in the absence of flurazepam¹². The broken lines represent antagonist potency in the presence of flurazepam 10^{-6} M, the dose ratios being obtained by comparison of the muscimol dose-response curves in the presence of flurazepam and flurazepam + antagonist. The slopes of the bicuculline lines were: continuous 1.070 ± 0.032 , broken line 1.008 ± 0.070 . The slopes of the picrotoxin lines were: continuous line 0.635 ± 0.021 , broken line 0.655 ± 0.049 . Comparisons of the intercepts of the lines²⁰ at the level (muscimol dose ratio - 1) = 4 indicated: a, no significant difference between the bicuculline lines ($P < 0.1 > 0.05$) at an equipotent bicuculline concentration ratio of 1.11 b, a significant ($P < 0.001$) reduction in the potency of picrotoxin in the presence of flurazepam 10^{-6} M at an equipotent picrotoxin concentration ratio of 2.67. These comparisons of antagonist potency are based on an assumption that bicuculline and picrotoxin did not antagonise the potentiating action of flurazepam (assumption (1) in the text). Re-calculation of the muscimol dose ratios by comparing control dose-response curves with those obtained in the presence of flurazepam + antagonist (assumption (2) in the text) resulted in a small additional displacement to the right of the Schild plots obtained in the presence of flurazepam. The lines remained parallel to those obtained in the absence of flurazepam and the equipotent antagonist concentration ratios increased to 1.57 for bicuculline and 3.80 for picrotoxin.

in the potency of picrotoxin. The data from binding studies also generally indicate that benzodiazepines and picrotoxin bind to separate sites^{8-11,15} although it has recently been found that benzodiazepines can reduce the binding of dihydropicrotoxin to brain membranes¹⁷. In Fig. 3, therefore, flurazepam is suggested to potentiate an endogenous process which facilitates or prolongs the opening of the ion channel and with which picrotoxin competes. Such an action of flurazepam could account for the small potentiation of muscimol. A change in the properties of the GABA receptor⁶ appears to be a less likely explanation since the potency of the GABA receptor antagonist bicuculline was little affected by flurazepam. The model does not

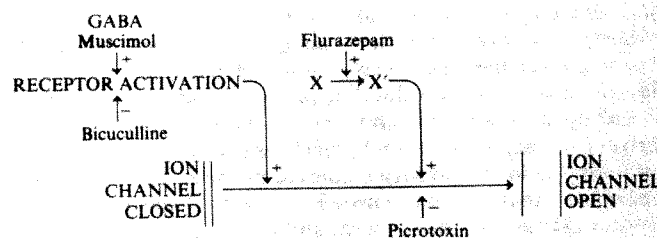


Fig. 3 Hypothetical model of the GABA response mechanism and the possible sites of action of some drugs. +, Agonist or facilitatory action, including antagonism of an inhibitory influence; -, indicates antagonism. $X \rightarrow X'$ represents an endogenous process which facilitates or prolongs channel opening. The intermediate steps between receptor activation and ion channel opening seem to be the minimum necessary to accommodate the pharmacological data from depolarisation responses to GABA and muscimol of afferent nerve fibres in the rat cuneate nucleus.

allow for an enhancement of benzodiazepine binding by GABA receptor activation⁸⁻¹¹, but there is now a possibility that this effect may involve a different population of GABA receptors from those mediating changes in membrane conductance and polarisation^{18,19}.

This model has been devised as the simplest arrangement which would accommodate all the drug effects on responses to GABA receptor activation reported here and which would also be compatible with the data from binding studies. Although the stages between GABA receptor activation and ion-channel opening are entirely hypothetical, they do have possible implications. The model suggests either (i) that flurazepam increases the duration of channel opening for each GABA receptor activation or (ii) that only a proportion of the GABA receptor activations result in channel opening and that flurazepam increases that proportion. It also seems that there is no requirement for a fixed ratio of benzodiazepine sites to GABA receptors in the neuronal membrane. It remains to be seen which of these predictions are correct, but it is clear that responses to GABA receptor activation can be modulated at a number of different sites.

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Calcium transients in mammalian muscles

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Contraction of vertebrate skeletal muscle is caused by calcium ions released from the sarcoplasmic reticulum (see refs 1, 2 for reviews). The ensuing transient change in the intracellular level of ionised calcium has been monitored using various Ca^{2+} indicators, such as murexide,³ aequorin^{4,5}, and arsenazo III^{6,7}. So far, most of what is known about these calcium transient derives from experiments on barnacle or frog muscle fibres, and it is desirable to extend such studies to mammalian muscle. We report here that the photoprotein aequorin^{8,9} can be used to monitor calcium transients in rat and human muscles, and that the transients decay more quickly in fast contracting muscle fibres.

The experiments were done on the fast-twitch extensor digitorum longus (EDL) and slow-twitch soleus (Sol) muscles of the rat, and on human intercostal muscle obtained during thoracic surgery. The isolated muscles were mounted in a bath perfused with oxygenated mammalian Ringer¹⁰, which contained $1-3 \times 10^{-7} \text{ g ml}^{-1}$ tetrodotoxin to abolish action potentials, and in most experiments 10 mM tetraethylammonium bromide was used to reduce delayed rectification. Muscle fibres were impaled

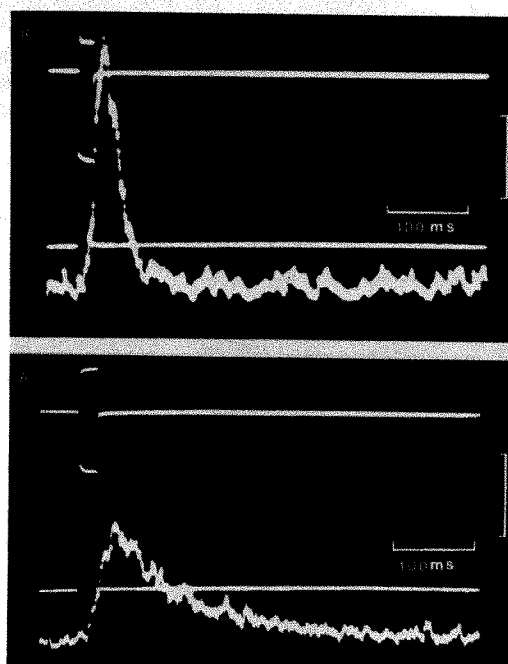


Fig. 1 Calcium transient in extensor digitorum longus (a) and (b) soleus muscle fibres of rat. The lowest traces show the aequorin responses, the middle ones record membrane potentials and the upper ones the clamp currents. Calibration bars are 2 nA for the light responses, 50 mV for the membrane potentials and 1 μA for the clamp current. Temperature, 25 °C. A time constant of 5 ms was used for the light recordings.

with two bevelled microelectrodes. One was filled with aequorin, dissolved in 120 mM KCl, 25 μM EDTA, 5 mM HEPES buffer (pH 7.9), and was used both for injecting aequorin into the fibre and for recording membrane potential. The other, inserted at a distance of 50-150 μm , was filled with either 3 M KCl or 4 M K-acetate and was used to pass current across the muscle fibre membrane to clamp the membrane potential, which was normally held at -80 mV. Aequorin was injected, under microscopic inspection by applying a pulse of pressure to the aequorin pipette. The light emitted, when Ca^{2+} combines with aequorin⁸, was recorded with a photomultiplier (EMI 9824) run at -900 V and placed about 1.5 cm above the surface of the muscle. Usually, the EDL and Sol were both mounted in the same chamber to standardise the experimental conditions.

When the muscle fibre membrane was strongly depolarised, a flash of light was recorded (Fig. 1), indicating a rise in the level of ionised calcium in the myoplasm. The Ca^{2+} is presumably released from the sarcoplasmic reticulum, since similar light responses could be recorded in Ca^{2+} -free solutions with 1 mM EGTA. Applying pulses of 20-60 ms duration, and graded intensity, to fibres held at -80 mV, light was first detected when the membrane potential was stepped to about -35 mV. This level was not very different from that at which a localised contraction in both fast and slow muscle fibres was first detected. However, in some cases the threshold depolarisation for light detection was higher than that for contraction, and the maximum light response was smaller. This probably occurred when the amount of aequorin injected by the pressure pulse was very small, or when some of the aequorin contained in the tip of the micropipette had been inactivated by the external Ca^{2+} , before insertion into the fibre. The amplitude of the light response with increasing depolarisations rose sharply at first and then tended to level off. Increasing the pulse duration increased the rise time, and amplitude of the light response. Thus, in many respects, the relationship between membrane potential and light response in mammalian muscles fibres resembles that obtained previously with arsenazo III⁶ and with aequorin (our unpublished data) in frog muscle fibres.

With depolarising pulse durations of a few milliseconds to 100 ms, the light response increased during the pulse and continued to rise well after the membrane potential had returned to the holding level (Fig. 1). A continued rise of the aequorin signal is also seen after the termination of iontophoretic pulses of Ca^{2+} discharged into droplets of aequorin¹¹. This suggests that the time during which calcium is released from the sarcoplasmic reticulum is shorter than the time to peak of the light response, and probably not much longer than the voltage clamp pulse. Presumably the depolarising pulse opens Ca^{2+} channels in the membrane of the sarcoplasmic reticulum and these channels, like those at motor nerve terminals¹², are rapidly closed once the membrane is repolarised.

The rate of rise and peak amplitude of the light response greatly depends on the amount of aequorin injected into the fibre. This makes it difficult to compare responses obtained in different muscle fibres, because it is difficult to load them always to the same degree. Nonetheless, it seems that the light response is greater in the fast EDL muscle than in the slow soleus. Preliminary examination of the relationship between membrane potential reached by the depolarising pulse, and the amplitude of the ensuing light response, indicates that the relationship is S-shaped in both fast and slow muscle fibres. The threshold for light detection is roughly the same for both types of muscles fibres, but thereafter the amplitude of the light responses to depolarising steps of increasing intensity rises more slowly, and attains a lower maximum, in the soleus fibres.

After reaching its peak, the light response decayed nearly exponentially, with a time constant that was essentially independent of the intensity and duration of the depolarising pulse. In analogy with previous results⁷ obtained in frog muscle, there was a marked difference in the rate of decay of the light responses of fast and slow muscle fibres of the rat. At 25 °C the decay time constant in EDL fibres was 16.0 ± 4.0 ms (mean \pm s.d. of 35 fibres from 14 muscles), while in Sol fibres, it was 42.4 ± 13 ms (mean \pm s.d. of 65 fibres from 13 muscles). With application of longer pulses, the light response decayed during the pulse. This decay was much slower than that after the end of a short pulse and is presumably caused by inactivation of the Ca^{2+} release mechanism from the sarcoplasmic reticulum, although other factors such as depletion of Ca^{2+} stores may be also involved. Again, the light in EDL fibres decayed more rapidly than in the soleus. The duration from half rise to half decay was 244 ± 64.6 ms (mean \pm s.d. of 8 fibres), while in Sol fibres it was 468 ± 118 ms (mean \pm s.d. of 16 fibres). The greater dispersion in the rate of decay of Ca^{2+} transients in soleus muscle fibres, may be related to the existence, in this muscle, of slow and 'intermediate' (10%) motor units¹³; but so far our results are inadequate to establish whether there are two populations of muscle fibres with clearly distinct Ca^{2+} transients. In both fast and slow muscles the light responses became slower with decreasing temperature. In the range between 15 and 40 °C, the time constant of decay changed more or less linearly with inverse temperature in Arrhenius plots in both muscles and the Q_{10} was

2.4 for EDL and 2.0 for Sol, but the difference of the Q_{10} value between EDL and Sol may not be significant considering the range of the values obtained at each temperature.

Calcium transients were also recorded from human intercostal muscle fibres; but their time course in the two samples examined so far was found to be very different. For instance, in one muscle (Fig. 2) the decay time constant of the aequorin light was 20.4 ± 3.0 ms (five fibres) while in a muscle from a different subject, it was 108 ± 22 ms (nine fibres). At present we do not know the cause of such a large difference.

The rate of rise and peak amplitude of the calcium transients in rat EDL muscle fibres was generally greater than in the soleus. This suggests that the rate of release of calcium from the sarcoplasmic reticulum may be greater in the fast fibres. Such a difference could arise from a corresponding difference in the number, or kinetic characteristics of the Ca^{2+} releasing sites in the reticulum of fast and slow fibres. The decay of the light responses reflects the time course of the processes which restore the intracellular calcium ion concentration to its resting level. It is not yet clear to what extent uptake of calcium by the sarcoplasmic reticulum is involved in determining the decay of the light responses. However, the marked difference in the rate of decay of light responses in EDL and Sol muscles is apparently in line with the observation that the sarcoplasmic reticulum is less developed^{14,15} and, when isolated, takes up calcium more slowly^{16,17} in the soleus muscle.

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Progressive inhibition of the Ca pump and Ca:Ca exchange in sickle red cells

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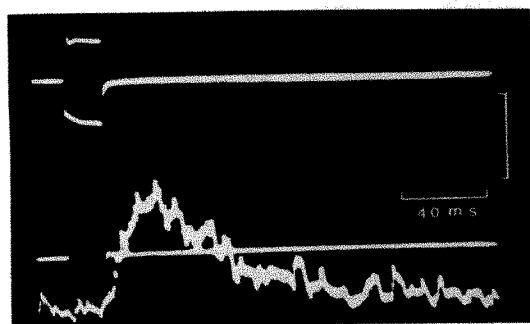


Fig. 2 Calcium transient in human intercostal muscle. Traces as Fig. 1. The calibration bar represents 2 nA for the light response, 50 mV for the potential and 1 μA for the current. Temperature, 25 °C. Time constant of light recording system, 5 ms.

Sickle cell anaemia red cells (SS) were reported to have a high Ca content and an increased Ca uptake on deoxygenation^{1,2}, but their Ca-pump activity was described as normal³. This seemed puzzling because the saturated Ca-extrusion rate of the normal, high Ca-affinity Ca pump is about 10 mmol per 1 cells per h (refs 3, 4) and the highest sickling-induced Ca influx reported in SS cells^{2,5} and observed in ATP-depleted sickle-trait (SA) red cells⁶ never exceeded 0.2 mmol per 1 cells per h. Normal pump performance is, therefore, incompatible with Ca accumulation unless SS cells have abnormally high Ca-binding capacity. We provide here evidence which suggests that SS cells have normal Ca-buffering capacity and probably genetically normal Ca pumps, but that the sickling process causes progressive Ca-pump failure and a marked reduction in Ca:Ca exchange.

Table 1 Cell-associated calcium pools in SS and normal (AA) red cells—total, ionophore mobilisable (free) and ^{45}Ca -tracer exchangeable in 2.5 h

Method	Conditions	Estimated calcium content of cells ($\mu\text{mol per 1 cells}$)	
		SS cells	AA cells
Atomic absorption spectroscopy	Washed in EGTA-free buffer	52.0 ± 8.2 (4)	11.6 ± 1.4 (3)
	Washed in EGTA-buffer	40.3 ± 17.2 (11)	5.7 ± 1.8 (7)
	After incubation with ionophore and EGTA	5.8 ± 2.3 (5)	$4.7, 5.4$ (2)
^{45}Ca influx	Washed in EGTA-buffer	4.5 ± 1.6 (5)	$1.3, 2.0$ (2)
	After incubation with ionophore and EGTA	0.26 ± 0.04 (4)	<0.20 (1)

For atomic absorption spectroscopy, red cells from fresh heparinised blood were washed four times with an ice-cold medium containing (in mM): NaCl, 140; KCl, 5; HEPES, 10 (pH 7.40); with or without EGTA, 0.1, and all buffy coat removed. Some samples were exposed to ionophore ($5 \mu\text{M}$) in the presence of EGTA at 37°C for 15 min before the final washes. After washing, the cells were lysed in distilled water, and haemoglobin precipitated with 5% trichloroacetic acid (TCA). Distilled water was deionised and gave a signal equivalent to less than $0.05 \mu\text{M}$ Ca. All the buffers used in the analysis were passed through Chelex 100 resin, TCA was redistilled following Harrison and Long¹⁵ and all plastic tubes and tips were rinsed with 5% redistilled TCA and deionised water before use. Atomic absorption spectroscopy was carried out on a Perkin-Elmer Model 360 spectrophotometer with an HGA 2100 graphite furnace, having a sensitivity of 15 pg Ca , and permitting accurate ($\pm 5\%$) measurement of $<0.5 \mu\text{M}$ Ca. Ca values (mean \pm 1 s.d.) are expressed in $\mu\text{mol l}^{-1}$ red cells, assuming a mean corpuscular haemoglobin concentration of 34 g dl^{-1} for the cells. The number of samples, each from a different patient, is shown in parentheses. Increasing the EGTA in the wash buffer from 0.1 to 5 mM did not lower the levels of red cell Ca by atomic absorption spectroscopy. The ^{45}Ca -tracer exchangeable calcium pool was estimated by measuring the influx of ^{45}Ca . Washed cells were resuspended at 10% haematocrit in the EGTA-free washing medium containing, in addition, $10 \mu\text{M}$ glucose and $1 \text{ mM } ^{45}\text{CaCl}_2$, and incubated for 2.5 h at 37°C . Subsequent washing and measurement of ^{45}Ca in the red cells were as described by Ferreira and V.L.L.⁸. The ^{45}Ca content of the cells was calculated by dividing the radioactivity per unit haemoglobin-estimated cell volume by the external specific activity of ^{45}Ca .

We first investigated the cytoplasmic Ca buffering of intact SS cells by the method of Ferreira and Lew^{3,7}. The equilibrium distribution of ^{45}Ca between cells and medium induced by a high concentration ($10 \mu\text{M}$) of the ionophore A23187 was measured with red cell suspensions (haematocrit $\sim 10\%$) from four sickle cell anaemia patients. The medium (A) contained (mM): NaCl, 75; KCl, 75; MgCl_2 , 0.2; HEPES-Na (pH 7.4), 10; glucose, 10, and two different initial concentrations of $^{45}\text{CaCl}_2$, of 0.020 and 0.100. At equilibrium, the SS cells contained 6.8–10.2 times more ^{45}Ca than equal volumes of suspension medium. The fraction of ionised Ca calculated from these values was 0.20–

0.29, falling within the lower part of the normal range (0.20–0.45)³.

It was important to determine whether the high original cell-associated Ca of the SS cells was free to equilibrate with added ^{45}Ca in the presence of the ionophore, so that the equilibrium distribution of the tracer would reflect the true distribution of the free Ca. Table 1 shows that addition of the ionophore A 23187 to EGTA-washed SS cells suspended in EGTA-containing media removed 86% of the cell-associated Ca. This indicated that most of the original cell-associated Ca was intracellular and free, and that it was available for equilibration with added ^{45}Ca in the presence of the ionophore.

There was a residual level of tightly bound, ionophore-resistant and non-exchangeable cell Ca, of about $5 \mu\text{mol per 1 cells}$, associated with both SS and normal cells. The surprising observation, however, was that in the absence of the ionophore, only about 10% of the free, ionophore-mobilisable Ca of SS cells exchanged with ^{45}Ca in 2.5 h, whereas all of the free, ionophore-mobilisable Ca of AA cells had equilibrated with the ^{45}Ca -tracer in that time. The tracer-equilibration rate of the SS cells is therefore at least 20 times slower than that observed in ATP-depleted AA cells⁸, the only conditions in which similar-size Ca pools can be maintained inside normal intact cells without ionophore or Ca-pump interference. The conclusion that Ca:Ca exchange must be extremely slow in at least some of the Ca-containing SS cells is further supported by efflux measurements from ^{45}Ca -preloaded SS cells. We see (Table 2, condition 4) a progressive reduction in the efflux of ^{45}Ca into a medium containing $1 \text{ mM } ^{40}\text{Ca}$ which, even after correcting for net ^{45}Ca release (from values in Table 2, condition 3), suggests a marked heterogeneity of exchange rates. After 2 h, the efflux of ^{45}Ca through all pathways combined had become negligibly small, whereas the cells still retained almost 22% of the initial ^{45}Ca pool. Furthermore, as there was no net Ca gain in these conditions (see Table 2, condition 3), all Ca movement across the membrane of those cells containing the residual ^{45}Ca pool, whether through pump, leak or Ca:Ca exchange, had virtually ceased.

We investigated Ca-pump function by following the release of ^{45}Ca from SS cells preincubated in the presence of $1 \text{ mM } ^{45}\text{CaCl}_2$ in the various experimental conditions described in Table 2. It can be seen (Table 2, conditions 2 and 3) that ^{45}Ca efflux is uphill, because it proceeds unchanged in the absence or presence of a large inward Ca gradient. The efflux, however, is two orders of magnitude smaller than that expected from a normal, high Ca-affinity pump saturated with internal Ca and ATP. As the ATP concentration per unit volume of cell water is apparently normal in SS cells, even in the dense cell fraction rich in irreversibly sickled cells⁹ whose Ca levels are particularly

Table 2 ^{45}Ca release from ^{45}Ca -preloaded SS cells

Condition	Pretreatment during ⁴⁵ Ca loading	Final incubation medium	⁴⁵ Ca content of cells at start of final incubation	⁴⁵ Ca efflux	Period during which flux measured (min)
			(μmol per 1 cells)	(μmol per 1 cells per h)	
1	Aerated, 90–180 min	Ca-free, 1 mM EGTA	2.9, 6.0 (2)*	0.6, 1.3	0–70
2	Deoxygenation, 90–180 min	Ca-free, 1 mM EGTA	19.2, 27.6 (2)	19.9, 20.6	0–10
3	Deoxygenation, 120 min	1 mM ⁴⁵ CaCl ₂	15.8 (1)	4.9, 5.7	10–70
4	Deoxygenation, 120 min	1 mM ⁴⁰ CaCl ₂	17.6 (1)	16.0	0–12
				3.6	12–50
				22.8	0–20
				8.7	20–40
				5.0	40–60
				2.1	60–90
				0.76	90–120

Red cells from fresh, heparinised blood were washed and resuspended (haematocrit, about 15%) in a medium similar to that used to measure cytoplasmic Ca buffering (medium A, see text) but containing $1 \text{ mM } ^{45}\text{CaCl}_2$. The cell suspensions were incubated under air (aerated) or 100% argon (deoxygenation) for the indicated times. After four washes in an ice-cold, Ca-free medium, the cells were resuspended in medium A at about 10% haematocrit and incubated at 37°C , under air, for up to 2 h. The presence in the suspension medium of EGTA, ^{40}Ca or ^{45}Ca of the same specific activity as that used during the loading period was as indicated. The ^{45}Ca content of the cells was calculated by dividing the ^{45}Ca activity per unit cell volume by the external specific activity of ^{45}Ca ; this calculation underestimates the true Ca content of the cells, because not all cell Ca had equilibrated with the tracer (see Table 1). It seems, however, a convenient way of expressing the results because it conveys some information on the approximate magnitude of the true Ca fluxes. The residual ^{45}Ca content of the cells in condition 4 after 2 h was $4.1 \mu\text{mol per 1 cells}$. Conditions 3 and 4 report the ^{45}Ca loss from the same cells in strictly comparable conditions.

* Mean values in each experiment. Number of experiments in parentheses.

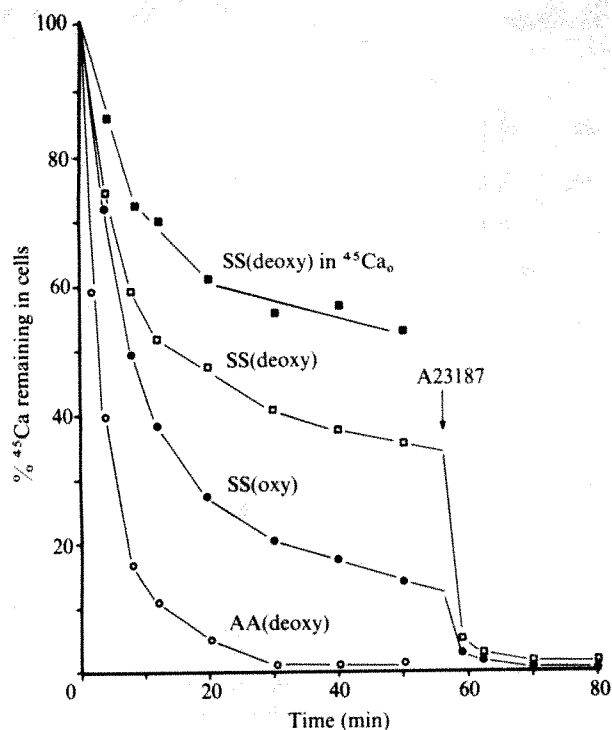


Fig. 1 Effect of a deoxygenation pulse on the percentual release of ^{45}Ca from normal (AA) and sickle (SS) red cells preloaded with ^{45}Ca in the presence of the ionophore A23187. Red cells from fresh heparinised blood were washed and resuspended at about 15% haematocrit in a medium of similar composition to that used to measure cytoplasmic Ca buffering (see text) but containing 1 mM $^{45}\text{CaCl}_2$. After 90 min incubation under air (oxy) or argon (deoxy), the ionophore A23187 was added to give a final concentration of 1 μM in the cell suspension, and the incubation continued for 90 s. The cells were then washed and resuspended at an haematocrit of about 10% in the same medium. In addition, each suspension also contained EGTA, 1 mM except for the one group noted to contain $^{45}\text{CaCl}_2$, 1 mM, with the same specific activity as that used during the loading period. The residual ^{45}Ca content of the cells was measured in the TCA supernatant of the washed cell lysate⁸. Ionophore (1 μM) was added after 55 min in two of the conditions with EGTA-containing media to show that the slowly extruded Ca pool had been retained by a permeability barrier, and was not tightly bound to cell components but was free to diffuse out of the cells. Comparison of the SS (deoxy) efflux curves in the absence and presence of ^{45}Ca shows that ^{45}Ca efflux represents uphill extrusion against a steep inward Ca gradient. The small difference between the two curves represents the full magnitude of the inward Ca leaks, including any residual ionophore-mediated permeability. The contribution these leaks could have made to the observed ^{45}Ca efflux would, therefore, have been unmeasurably small. The initial ^{45}Ca content of the cells at the beginning of the final incubation and designated 100% in the curves shown was (in $\mu\text{mol per 1 cell}$): AA (deoxy), 436; SS (oxy), 187; SS (deoxy), 74.6. In a similar experiment, not shown here, the ^{45}Ca content of SS (oxy) cells fell from 51.6 to 5.8 μmol and per 1 cells in 45 min and that of SS (deoxy) cells fell from 35.1 to 12.9 μmol per 1 cells in the same period. The percentual changes were almost identical in the two experiments despite the different initial ^{45}Ca -content values.

high^{1,2}, the low Ca-extrusion rate must reflect an abnormal response of the Ca pump itself.

The nature of this Ca-pump failure was investigated by experiments similar to those described in Table 2, except that 60–90 s before the end of the preincubation the cells were more uniformly loaded with ^{45}Ca by a brief exposure to the ionophore A23187. After washing away the ionophore^{10,11}, the release of ^{45}Ca was investigated as shown in Fig. 1. We see that the ionophore allowed ^{45}Ca entry into a population of SS cells with normal Ca pumps, for the initial extrusion rate was similar in SS and normal cells. In contrast to normal red cells, however, a substantial fraction of the SS cell population exhibited weak Ca-pump activity. Furthermore, following a sickling pulse, either the fraction of weak pumping cells or the size of the Ca pool inside these cells, or both, seemed to be substantially increased.

Because some of the rapidly extruded Ca must have come from cells that had been sickled during the preincubation, a single sickling episode is not always sufficient for Ca-pump

inhibition. The mechanism of inhibition is unknown but it could involve irreversible dissociation of calmodulin from the inner pump surface, as observed with the $(\text{Mg}^{2+} + \text{Ca}^{2+})\text{ATPase}$ of SS ghosts¹². Inaccessibility of calmodulin, and even Ca, to their receptor sites on the Ca pump might result from a shielding effect by the large amount of membrane-bound, denatured haemoglobin S which has been observed in those ghosts^{13,14}.

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Nitrogen-fixing growth by nonheterocystous cyanobacterium *Plectonema boryanum*

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Many oxygen-evolving nonheterocystous cyanobacteria can synthesise nitrogenase^{1,2}, but only a few are able to protect the enzyme from inhibition and denaturation by oxygen³⁻⁵. It is difficult to reconcile concomitant oxygenic photosynthesis and the obligately anaerobic process of nitrogen fixation in the remaining strains. One possibility is a temporal separation of the processes: *Plectonema boryanum* (U. Tex strain 594) has been shown⁶ to fix nitrogen and then grow oxygenically to the limits of the fixed nitrogen when incubated without combined nitrogen under N_2/CO_2 . Growth was not extensive. Here conditions are described in which the same strain of *P. boryanum* will simultaneously fix nitrogen and grow extensively, fully pigmented, apparently with sufficient metabolic energy to drive nitrogen fixation.

Preformed or exogenous carbohydrate is necessary for the expression of nitrogenase in strains such as *P. boryanum* when the enzyme is induced under N_2/CO_2 (ref. 2). Some cyanobacteria seem to prefer anoxic, reducing environments⁷, and some strains are capable of anoxygenic photosynthesis, oxidising hydrogen sulphide to sulphur⁸. *P. boryanum* is incapable of anoxygenic CO_2 fixation⁹, may be able to oxidise hydrogen sulphide to sulphur¹⁰, and will grow heterotrophically¹¹. It seemed that added carbohydrate might substitute for the lack of CO_2 fixation and that reducing conditions might inhibit oxygenic photosynthesis⁸ or chemically reduce free oxygen and thus enable nitrogen fixation and growth to occur simultaneously. Colony growth was obtained on fructose-supplemented plates

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incubated in an anaerobic jar into which an atmosphere of 95% $N_2/5\%$ CO_2 was introduced through repeated partial evacuation, and then sulphide was added by injecting an Na_2S solution. The jar was maintained free of O_2 by a solution of sodium dithionite. Green growth required light, fructose, nitrogen gas and a limited amount of sulphide. A sulphide-stat was then constructed in which the N_2/CO_2 was first bubbled through a constantly refreshed sulphide solution so that the carry-over of sulphide into the culture medium maintained a constant sulphide concentration in the range of $5-10 \times 10^{-6}$ M. This facilitated detailed examination of nitrogenase activity and concomitant growth by the cyanobacterium.

Figure 1 shows some of the features of nitrogen-fixing growth by *P. boryanum*. After a lag of less than 1 day (some experiments had longer lags), growth proceeded logarithmically with a doubling time of about 24 h. On day 5 the culture began to lose colour, but logarithmic growth and full pigmentation were restored by the addition of fructose. The cyanobacterium would grow without a break in logarithmic growth on 0.2% fructose; Fig. 1 thus demonstrates a clear requirement for fructose. The specific activity of nitrogenase (as acetylene reduction) showed a transient peak after induction (similar to that seen following the induction of nitrogenase in *Gloeocapsa*³), reaching a specific activity of almost 5×10^{-8} M ethylene per min per mg protein, one of the highest reported for a growing cyanobacterium. Total

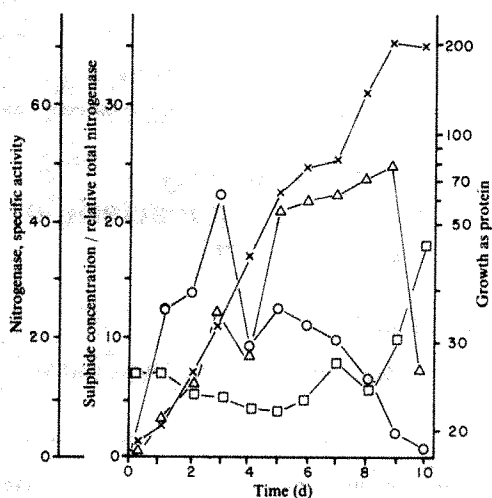


Fig. 1 Growth and nitrogen fixation by *P. boryanum* in the sulphide-stat. *P. boryanum* was grown under N_2/CO_2 in BG-11 (ref. 12) plus $NaNO_3$, collected and washed twice with BG-11 minus combined nitrogen, and then inoculated into the sulphide-stat. The growth vessel contained 400 ml BG-11 medium minus combined nitrogen plus 0.1% fructose buffered with $5/10^{-3}$ M N-Tris (hydroxymethyl) methyl-2-amino methane sulphonic acid, pH 7.8, and was maintained at $30^\circ C$ under 2,500-lux incident illumination. At a rate of 50 ml min^{-1} , 95% $N_2/5\%$ CO_2 was fed through a constant 500 ml of an anaerobic solution of 10^{-1} M-Tris (hydroxymethyl) amino methane, pH 7.4, 10^{-1} M sodium dithionite (which was refreshed at a rate of 4 ml h^{-1} by a similar solution containing 10^{-1} M Na_2S) and thence into the growth vessel. Strict anaerobiosis was maintained in the culture vessel and in nitrogenase determinations. Growth (x), scale units $10^{-6} \text{ g ml}^{-1}$, was monitored as protein by the Lowry *et al.*¹³ method as before¹⁴. Nitrogenase was assayed by the acetylene reduction technique¹⁵ as before¹⁴, except that the assay was performed in 10-ml plastic syringes (No. 7370, Pharmaseal Labs, Glendale), sealed with a piece of soft, translucent Teflon. Sodium dithionite (final concentration 2 mM) was added to each sample as a precaution against oxidation. Nitrogenase is expressed as relative total activity (Δ) (proportional to the ethylene evolved per min per ml), and as specific activity (\circ) (10^{-9} mol C_2H_4 per min per mg protein). Sulphide (\square), scale units 10^{-6} M, was measured using an Orion 94-161 electrode and antioxidant techniques described in Orion manual 94-161M (Orion, Inc., Cambridge, Massachusetts). The timescale (ordinate) is in days; parameters were measured at the same time each day. On day 5 de-aerated 10% fructose was injected to an additional 0.1% final concentration.

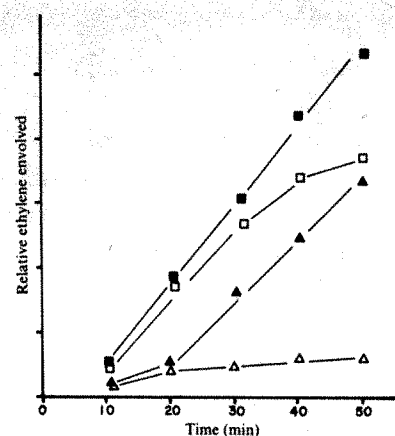


Fig. 2 Dithionite stimulation of nitrogenase activity (acetylene reduction) in cells grown in a sulphide-stat (\blacksquare , \square) and cells incubated without sulphide (\blacktriangle , \triangle). Open figures, no dithionite added to assays; closed figures, dithionite added (as in Fig. 1). Ordinate, time after addition of acetylene and dithionite. Abscissa, relative amount of ethylene evolved at the time indicated. Assayed as described in the legend to Fig. 1.

nitrogenase activity increased throughout the growth cycle. This is the first demonstration of concomitant logarithmic growth and nitrogen fixation by a blue-green bacterium lacking a cellular oxygen-protective mechanism. The sulphide concentration remained in the range of $5-8 \times 10^{-6}$ M until the end of the experiment when there was an increase. Except for the transient period between day 5 and day 7 the culture remained fully pigmented. A control culture without sulphide showed some growth, but with a much lower nitrogenase activity and with an almost complete lack of phycobiliproteins (as 625 nm absorbance). Figure 2 shows that the nitrogenase activity (acetylene reduction) in such a control culture was noticeably stimulated by added sodium dithionite, as demonstrated for similar cultures without fructose¹², whereas activity in a culture grown in a sulphide-stat was not. Thus, the sulphide-stat facilitates logarithmic, nitrogen-fixing, photoheterotrophic growth with complete pigmentation and sufficient metabolic energy to drive nitrogenase.

These observations suggest natural flexibility in the metabolism of *P. boryanum*. Presumably, carbohydrate replaces the carbon fixed through photosystem II-driven reactions and cyclic photophosphorylation generates ATP. The presence of most major pigments would suggest that photosystem II may be at least partially active.

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Low molecular weight circular and linear DNA in mitochondria from normal and male-sterile *Zea mays* cytoplasm

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Maternally inherited cytoplasmic variation in plants is well documented¹. One consequence of such variation in maize (*Zea mays* L.) is male sterility, sources of which have been classified into three groups (T, S and C) depending on nuclear gene fertility restoration²⁻⁴. These groups and the normal (N) male-fertile cytoplasm can be differentiated by restriction endonuclease analysis of mitochondrial DNA (mtDNA)^{5,6}, although differences have also been found within the N and C groups^{7,8}, and mitochondrial translation products^{9,10}. Encouraged by the finding that mitochondria of S-cytoplasmic types contain discrete small DNA species¹¹, we have looked for low molecular weight DNAs in mitochondria from plants with N, T, C and S cytoplasm. We report here that all four cytoplasm types contain supercoiled circular DNA molecules of approximately 1,940 base pairs. This is the first small supercoiled circular DNA to be found in higher plants. Its structure suggests it may be an autonomously replicated plasmid. N, C and S cytoplasm also contain a DNA species of approximately 2,350 base pairs which is not present in T cytoplasm. C cytoplasm contain two additional circular DNA species of 1,570 and 1,420 base pairs.

Figure 1 shows an electrophoretic analysis of mtDNA from the different cytoplasmic types. All bands are resistant to RNase but sensitive to DNase. There are three DNA bands which are common to all cytoplasm. These are the supercoil (CCC), open-circle (OC) and linear (L) conformations of the same sequence (Fig. 1). Evidence for such a relationship between the three bands is: (1) S₁ nuclease treatment of CCC DNA produced OC and L molecules; (2) cRNA probes¹² of OC DNA hybridised to CCC, OC and L bands of mtDNA extracts on Southern blots¹³; and (3) electron microscopy¹⁴ showed the DNA from the CCC and OC bands to consist of circular, double-stranded molecules (Fig. 2a, b). The CCC conformations of the molecules were too small for positive identification in the electron micrographs but, during purification of the CCC DNA from the preparative gel and/or the spreading technique for electron microscopy, some molecules nicked to produce OC and L forms (46% were circular) (Fig. 3c, d). Similarly, some linear molecules were formed from DNA in the OC band (40% were circular) (Fig. 3a, b). The CCC and OC bands have a similar mean length of approximately 1,940 base pairs (assuming the Φ X174 standard to be 5,380 base pairs¹⁵) (Fig. 3a, c). We conclude that this common DNA species exists, in mitochondria, as a CCC conformation which nicks to OC and L forms during mtDNA isolation.

Mitochondrial DNA of T cytoplasm plants lack the 2,350-base pair DNA species found in N cytoplasm (labelled as 'T' in Fig. 1). N and T lines from nine different nuclear backgrounds were compared and all produced the characteristic N or T pattern in Fig. 1. Mitochondrial DNA preparations from HA, RS and Q cytoplasm also had the characteristic T pattern, indicating that these cytoplasm are members of the T group^{3,4,10,16}. Six lines possessing T cytoplasm restored to male fertility by nuclear restoring genes produced the same pattern as male-sterile T types (Fig. 1, TRf track). Mitochondrial DNA was also obtained from two T seed sources which had been regenerated from callus tissue selected in the presence of *Helminthosporium maydis* race T toxin^{17,18}. The regenerated

plants were 100% male-fertile and fully resistant to the fungal toxin. However, their mtDNA produced the characteristic DNA banding pattern of T male-steriles.

S-group cytoplasm possess two linear mtDNA species not found in other cytoplasm (labelled 'S' in Fig. 1 and as described previously^{11,16,19}). S cytoplasm restored to male fertility by nuclear restoring genes show the same pattern as S male-steriles^{11,19}.

C types possess two DNA species, not found in other cytoplasm (labelled 'C' in Fig. 1). These DNA species were found in C types of the four different nuclear backgrounds examined, in RB cytoplasm which is a member of the C group^{3,4,10,16}, and in the one C cytoplasm line examined which had been restored to male fertility by nuclear restoring genes (Fig. 1, CRf track). Electron microscopic analysis¹⁴ of the C-specific DNA species showed the presence of double-stranded, circular molecules (Fig. 2c, d). The mean size of these circular molecules was about 1,570 base pairs for the slower migrating species (19% were circular) and about 1,420 base pairs for the faster migrating species (23% were circular) (Fig. 3e-h). Southern blot hybridisations¹³ showed that there is no sequence homology between the circular DNA species common to all cytoplasm described above and the DNA species specific to S or C types.

Although our data do not demonstrate a direct relationship between the presence of these low molecular weight species and male sterility, several lines of evidence suggest a specific involvement of these DNA species in such sterility. Not only is the T-type cytoplasm the only male-sterile cytoplasm to lack a DNA band when compared to N but, significantly, plants carrying T cytoplasm exhibit the most extreme expression of male sterility of all the three male-sterile cytoplasm. Plants carrying S and C cytoplasm, however, possess additional mtDNA

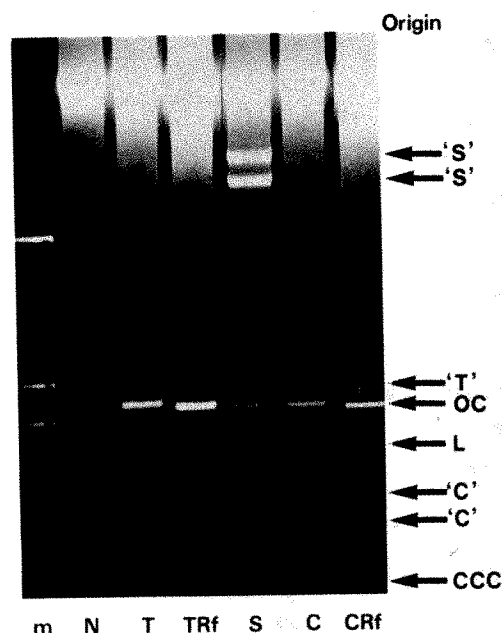


Fig. 1 Electrophoresis on 1.5% agarose gel of mtDNA preparations from N, T, TRf (T restored), S, C and CRf (C restored) cytoplasm in B37 nuclear background. 'm' is a marker track of bacteriophage λ DNA digested with *Hae*III, the sizes of the bands are 3.9, 2.4, 2.1, 1.7 and 1.19 kilobases. 'S', 'T' and 'C' indicate band differences pertinent to S, T and C cytoplasm respectively. CCC, OC and L indicate supercoil, open-circle, and linear bands, respectively. Etiolated shoots were grown, mitochondria purified, mtDNA isolated and electrophoresis carried out exactly as described elsewhere¹⁶. Cytoplasmic sources used were; N, T and TRf carried in A73, W59M, A495, W33, B37, B9A nuclear backgrounds. N and T were also used in CO192 \times WJ, WF9 and F7 \times F2. HA, RS and Q were in CO192 \times WJ. C was in B37, WF9, A632 and CO192 \times WJ. CRf was in B37. RB was in CO192 \times WJ. S was in W13, W117, B37, WF9, CO192 \times WJ. SRf was in B37. Eighteen members of the S group of cytoplasm were used in CO192 \times WJ.

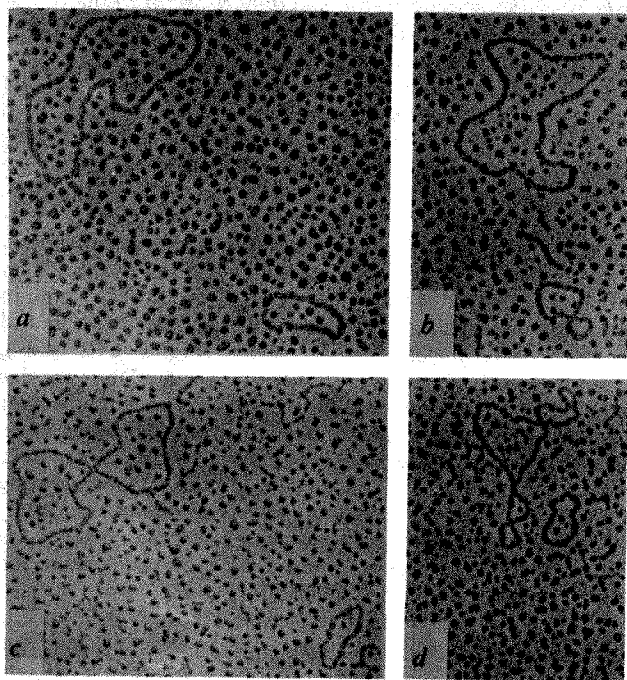


Fig. 2 Low molecular weight circular DNA molecules from mitochondrial preparations of C cytoplasm in CO192 × WJ nuclear background. DNA bands were isolated from a preparative agarose gel. *a*, OC band (see Fig. 1); *b*, CCC band; *c*, slower migrating 'C'-specific band; *d*, faster migrating 'C'-specific band. The large circular DNA molecule in each frame is ΦX174 bacteriophage included in each spread as an internal standard.

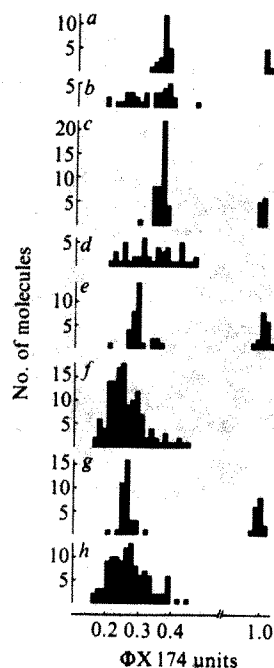


Fig. 3 Size-frequency distribution of DNA molecules from mitochondrial preparations as in Fig. 2. *a*, Circles from OC band (see Fig. 1); *b*, linears from OC band; *c*, circles from CCC band; *d*, linears from CCC band; *e*, circles from the slower migrating 'C'-specific band; *f*, linears from the slower migrating 'C'-specific band; *g*, circles from the faster migrating 'C'-specific band; *h*, linears from the faster migrating 'C'-specific band. The size distribution of the ΦX174 molecules used as internal standards with each spread is also shown. Photographic negatives of the electron micrographs were enlarged and the length of the DNA molecules measured with a map measure.

species not found in each other or N and T types and frequently exhibit only incomplete male sterility. In fact, some S types have reverted to complete fertility in the absence of any known nuclear restoring genes²⁰.

This finding of six different kinds of low molecular weight DNA molecules in maize raises many interesting questions in addition to their role in pollen fertility. It is clearly important to investigate their origin and their mode of replication. We are now studying their sequence relationship to other DNAs of the cell. If they are self-replicating they are potentially very useful tools for the study of plant transformation.

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A plasmid associated with virulence in the marine fish pathogen *Vibrio anguillarum* specifies an iron-sequestering system

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Many of the high-virulence strains of the marine fish pathogen *Vibrio anguillarum* isolated from epizootics of the widespread fish disease vibriosis, harbour a specific plasmid class which is absent from low-virulence strains¹. Curing experiments have confirmed a link between this specific plasmid class and the ability of *V. anguillarum* to establish infections². In general, all bacterial virulence factors promote growth in the antagonistic environment of the host defence mechanisms. One line of defence is provided by the proteins transferrin and lactoferrin³⁻⁵, which bind iron, rendering it unavailable to pathogens. A mechanism whereby invading bacteria may successfully compete for the otherwise unavailable iron could therefore become crucial in enabling them to proliferate in body fluids and tissues. I report here evidence which shows that the *V. anguillarum* virulence plasmid specifies a very efficient iron-sequestering system enabling bacteria to survive in conditions of limited iron availability.

To determine whether a plasmid-mediated iron sequestering mechanism exists in *V. anguillarum*, the growth kinetics of selected *V. anguillarum* biotype I⁶ strains of high virulence and their cured low virulence derivatives were studied in minimal

Table 1 Correlation between presence of plasmid, ability to grow in presence of transferrin and virulence of strains of *Vibrio anguillarum*

<i>V. anguillarum</i> strain	Presence of plasmid	MM	Generation time (h) in MM+transferrin	MM+transferrin + iron	Virulence (LD ₅₀)
775(pJM1)	pJM1	1.0	1.1	1.1	1.0 × 10 ³
775 (pJM11)	pJM11	1.0	1.2	1.0	3 × 10 ³
LS173(pJM1)	pJM1	0.9	1	1.0	2 × 10 ³
133S(pJM1)	pJM1	1.1	0.8	0.9	1.3 × 10 ³
H-775-850	pJM11	1.0	0.9	1.1	0.9 × 10 ³
E-775-300	pJM11	1.1	1.0	1.0	1.5 × 10 ³
H-775-1	No plasmid	1.1	4.0	1.2	2.1 × 10 ⁷
E-775-100	No plasmid	1.0	3.5	1.0	2 × 10 ⁶
286D	No plasmid	1	2.7	1.0	1 × 10 ⁸
NCMB1291	No plasmid	0.8	2.9	1.1	2 × 10 ⁷

Clones were analysed for plasmid DNA by agarose gel electrophoresis as before⁸. pJM11 was generated by transposition of the TnA sequence containing the ampicillin resistance genes to the original *V. anguillarum* virulence plasmid pJM1 (ref. 2). Growth curves in minimal medium (MM), MM+transferrin (2.3 µM) or MM+transferrin (2.3 µM)+iron (0.2 mM FeCl₃) were obtained as described in Fig. 1. Generation times were calculated on the portion of growth curves between 6 and 14 h of growth at 22 °C. Virulence was tested on juvenile coho salmon (*Oncorhynchus kisutch*) weighing about 14 g as before¹. Virulence is quantified as LD₅₀ values (number of microorganisms that will kill 50% of the animals inoculated) as determined by the Reed-Muench method⁹.

medium in the presence of transferrin. This protein binds the trace amounts of iron present as an impurity (about 3 µM) in minimal medium, making it unavailable for bacteria growth. The strains used were the plasmid-containing, high virulence 775 (pJM11) and its isogenic low virulence derivative H-775-3 obtained by curing the pJM11 plasmid from 775 (pJM11) at 37 °C, a temperature higher than its optimal growth temperature².

Figure 1a shows that addition of transferrin (final concentration 2.3 µM) to the culture medium inhibited the growth of the low virulence plasmid-less H-775-3 strain. Addition of excess iron (as 0.2 mM FeCl₃) to the medium totally reversed the inhibitory effect of transferrin by saturation of the iron-binding sites on the protein molecule. Similar results were obtained with other cured derivatives obtained by either exposure to 37 °C (H-775-1) or to ethidium bromide (E-775-100) (Table 1). Figure 1b shows the growth kinetics for the high virulence parent strain 775 (pJM11) containing the virulence plasmid. Growth of this strain is not affected by the concentration of transferrin which is inhibitory for the cured strains. Table 1 shows that those clones which were exposed to 37 °C (H-775-850) or to ethidium bromide (E-775-300) during the curing treatments but retained the plasmid still had a high virulence phenotype. It also shows that they could grow in the presence of transferrin, indicating that the curing procedures had not modified the pathogenic properties or ability to utilise complexed iron of bacteria other than in those cured of their pJM11 plasmid. Analysis of other high and low virulence strains isolated from different locations¹ showed similar correlations between segregation of pJM11, attenuation of virulence and inability to grow in the medium with transferrin (Table 1). My results thus suggest that the *V. anguillarum* virulence plasmid specifies a very powerful iron sequestering system which enables *V. anguillarum* to utilise highly complexed iron *in vitro*.

Is the possession of this plasmid-mediated iron uptake system a selective advantage *in vivo* for *V. anguillarum* infections? The results in Table 2 strongly support this contention. In experimental infections of fish with low-virulence cured derivatives of *V. anguillarum*, the mean lethal dose values (LD₅₀) are decreased about 300-fold if iron is included in the inocula, while added iron does not affect the LD₅₀ value in infection with the plasmid-containing strain 775 (pJM11). Thus there is a greater selective advantage for the plasmid-containing strain only when iron is not included in the inoculum. These results, of course, do not preclude the existence of other chromosomal or plasmid-mediated virulence determinants, which can play a part in other stages of the invasion process. However, considering the results reported here, it is possible to speculate that breeding and selection for transferrin genotypes of salmonids which are more resistant to the *Vibrio anguillarum* plasmid-mediated iron

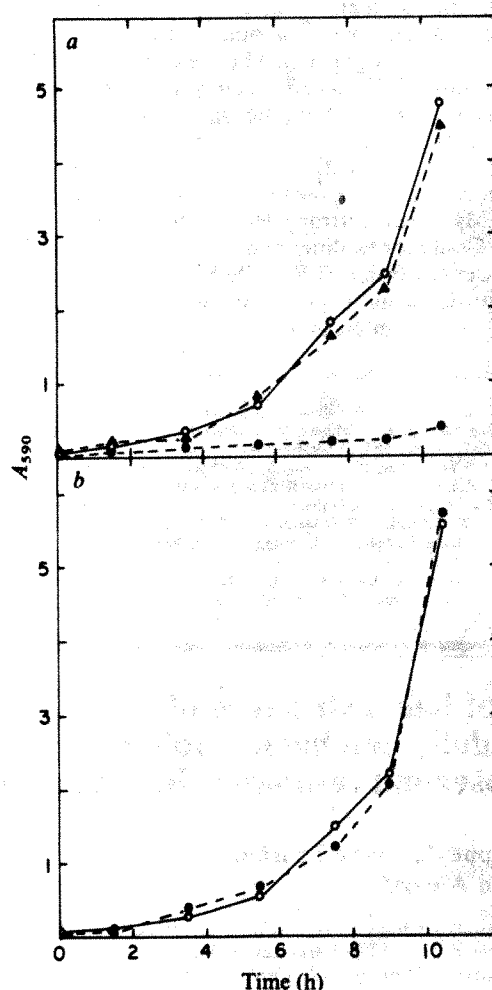


Fig. 1 Effect of transferrin on the growth rate of low virulence (a) and high virulence (b) *V. anguillarum*. Growth curves were obtained for: ○, minimal medium; ●, minimal medium plus 2.3 µM transferrin (Sigma); ▲, minimal medium plus 2.3 µM transferrin and 0.2 mM FeCl₃. Bacteria were grown overnight at 22 °C in a glucose-casamino acids (Difco) M9 salts minimal medium. Those cultures were used to inoculate sets of 250 ml flasks containing 100 ml of fresh similar medium at 22 °C with the different additions. Absorbance at 590 nm (*A*₅₉₀) was measured using a Gilford-vacuum micro-sample spectrophotometer. Samples were withdrawn every 2 h and the *A*₅₉₀ was determined. a, Low virulence H-775-3 strain obtained by curing plasmid pJM11 (Ap^r) from 775 (pJM11); b, high virulence *V. anguillarum* 775 (pJM11).

Table 2 Effect of iron on experimental infections of fish with cured low-virulence strains of *V. anguillarum*

Strain	LD ₅₀ control	LD ₅₀ with iron	Increase in virulence
H-775-3	2.1×10^7	8.2×10^4	256-fold
H-775-7	3.2×10^6	8.7×10^3	368-fold
E-775-100	2.1×10^6	9.0×10^3	233-fold
775 (pJM11)	4.3×10^3	3.3×10^3	1.3-fold

Control LD₅₀ values were determined as described in Table 1. LD₅₀ values with iron were determined by including 86 µg iron (as ferric ammonium citrate) in the bacterial inoculum. In all cases, inocula were injected subcutaneously (0.1 ml) behind the fish dorsal fin. No controls, into which 86 µg iron (as ferric ammonium citrate) in saline solution was injected, died.

sequestering system could open new avenues to the control of fish disease. Differences in susceptibility of salmonids with different transferrin genotypes have previously been shown in infections with the causative agent of bacterial kidney disease⁷. Experiments on this trend as well as the characterisation of the iron sequestering system in *Vibrio anguillarum* are in progress.

Williams has described¹⁰ another plasmid-mediated iron-uptake system as being specified by the Col V plasmid of those invasive strains of *Escherichia coli* associated with cases of bacteraemia in man and domestic animals. It would be interesting, both from an evolutionary as well as an epidemiological standpoint to assess whether the *V. anguillarum* and *E. coli* plasmid-mediated iron uptake systems are related.

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Study of haem structure of photo-deligated haemoglobin by picosecond resonance Raman spectra

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It is well known that the oxygen affinity of haemoglobin depends on the number of combined oxygen molecules¹. This cooperative effect is considered to arise from a reversible protein transition between two forms which differ in tertiary and quaternary structure². However, the various steps of the structural changes concerning the protein and the haem have not been identified. Using time-resolved spectroscopy coupled to flash photolysis, we have attempted to elucidate the influence of protein on the relaxation processes of haem in haemoglobin. We now report our first results obtained in a picosecond time-resolved resonance Raman study of haemoglobin.

Resonance Raman scattering from haemoglobin solution was induced by light pulse at 532 nm obtained by second harmonic generation from a YAG modelocked Quantel laser³. Two different temporal pulse lengths— 15×10^{-9} and 30×10^{-12} full width at half maximum (FWHM)—were used; the maximum energy delivered per pulse was 300 mJ and 40 mJ, respectively. By means of a cylindrical lens focusing⁴ (focal length = 750 mm), the laser beam was passed through an aperture (0.2 mm) to the haemoglobin sample. The cell (10×20 mm) containing the sample was put into another cuvette (30×30 mm) containing the same buffer as the sample. The laser beam arrived at normal incidence on one side of the first cell and approximately 30° to one side of the sample cell. This arrangement gives a good scattered signal at 90° from the incident beam, permitting the use of lower laser power and consequently, reducing the reflection. The residual Rayleigh scattering of the sample was reduced by placing an interference filter in front of the entrance slit of a polychromator (used in the first order) with an 1,800 grooves mm⁻¹ holographic grating. The detection system was similar to that used by Bridoux and Delhay⁵. The photocathode of a front stage image intensifier tube (EMI 9912) was located in the focal plane of the spectrograph. This system can give a light gain of 10^6 at the maximum photocathode sensitivity. We have chosen a gain of 10^5 . The picture on the target outlet of the intensifier tube was taken up by a vidicon SEC camera (EMR). The spatial resolution of the system was 5 lines mm⁻¹, with a sensitivity equivalent to $ASA \times 10^8$. The spectral information was obtained in a multi-channel analyser system (Didac 800) and recorded in an X-Y plotter. In all cases, to eliminate back-lash effects in the grating, the spectrometer was calibrated using the 586.0, 583.4, 580.3 and 573.9 nm lines of an argon lamp. For the recording of the spectra the input slit was set at 0.2 mm, corresponding to a theoretical spectral resolution (FWHM) of 4 cm⁻¹. The technical arrangement is shown in Fig. 1.

Haemolysates were extracted from fresh human adult blood, and membrane-free samples were obtained by centrifugation at 18,000 r.p.m. (ref. 6). Haemoglobin A was stripped of ions on a mixed-bed ion-exchange resin AG501 × 8 (Biorad)⁷. The oxygenated haemoglobin solution was buffered in 0.1 M potassium phosphate at pH 7. Deoxyhaemoglobin (Hb) was obtained by addition of a small fraction of sodium dithionite into buffered oxygenated haemoglobin (HbO₂). At room temperature Hb was converted to the carbon monoxide complex (HbCO) by using CO gas at a pressure of 1 atm above the solution. During the experiments the temperature was held at 22 °C. All solutions for the laser runs were at a concentration of 3×10^{-3} M calculated on the basis of haem. As the optical density of the sample is very high ($A_{1\text{cm},532} = 35$), the weak peak intensity (6 GW cm⁻²) used from the picosecond flash laser cannot initiate stimulated processes in the haemoglobin solution. Nevertheless, in the same optical geometric conditions, picosecond spectra show a background noise higher than that of nanosecond spectra. This noise may be due to energy dissipation processes of the electronically excited haem states⁸ produced by the high-power density laser of the picosecond pulse.

Nanosecond time-resolved resonance Raman spectra represent an average result of several laser shots (15–20 laser pulses). However, each picosecond time-resolved resonance Raman spectrum was obtained with the accumulation of 100 laser pulses (at least 2 s between each pulse). In these conditions, artefacts with repeated picosecond laser pulses are avoided because haemoglobin is always completely religated in less than 1 s. Not all spectra were smoothed out or normalised for intensity.

A typical transient picosecond resonance Raman spectrum of the haem structure-sensitive spectral region (1,300–1,700 cm⁻¹) of haemoglobin is shown in Fig. 2b. Both direct haem-protein interaction, which should change the porphyrin backbone symmetry, and iron electronic states variations, alter most of the stretching frequencies of C–C and C–N bonds in the porphyrin ring which are seen in the Raman spectrum range between 1,300

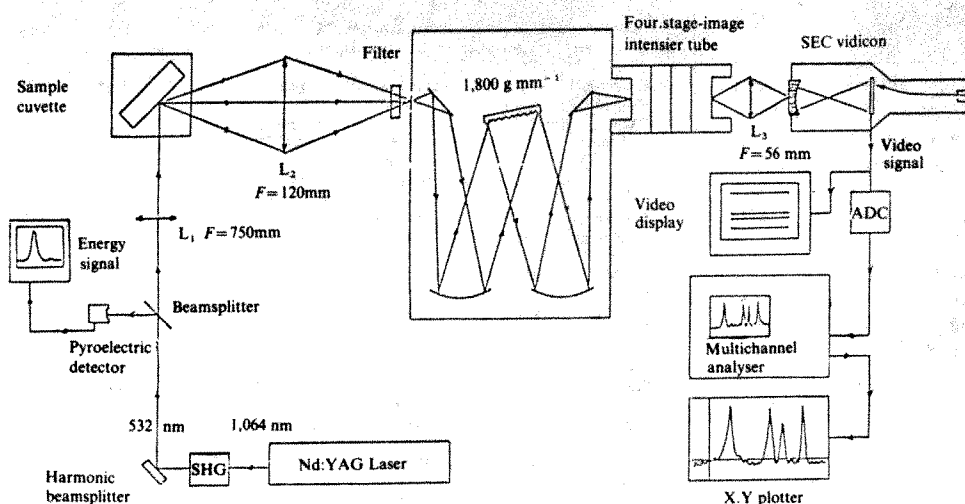


Fig. 1 Experimental arrangement.

and $1,700\text{ cm}^{-1}$ (refs 9–12). This spectrum seems to be the superposition of spectra of HbO_2 and stable deligated haemoglobin (Hb) (Fig. 2) and reflects the general pattern observed with nanosecond Raman spectra.

Relative amplitude changes between spectral bands have not been studied and correlated with laser energy. However, no laser pulse intensity dependence has been detected in the frequency positions of the transient picosecond haemoglobin spectra with an excitation wavelength of 532 nm, the frequencies of the observed bands were $1,586 \pm 2$ and $1,640 \pm 2\text{ cm}^{-1}$ for oxyhaemoglobin and $1,554 \pm 2\text{ cm}^{-1}$ for deoxyhaemoglobin. This result suggests that a Hb-like photoproduct was generated from HbO_2 , and that this photoproduct appeared in 30 ps.

The oxygen photo-deligation process is complete within 2.5 ps (ref. 13) and is initiated with the protein in the oxygenated structure (*R* configuration). A recent study of the photodissociation of HbCO by time-resolved absorption spectroscopy indicates that the iron–ligand linkage break leads to a readjustment of the haem in its protein pocket. This haem–protein readjustment occurs in microseconds¹⁴. The evolution to a more stable quaternary *T* state seems to take longer¹⁵. Also, changes in porphyrin structure caused by deligation and detected by picosecond time-resolved resonance Raman spectra cannot be associated with the protein constraints exerted on the haem; these include the imidazole strain and Van der Waals contacts. Thus, the recent finding that only a very short iron motion exists between oxy- and deoxyhaemoglobin¹⁶ confirms that the differences between the resonance Raman spectra of these compounds depend primarily on the iron coordination states (liganded or not).

From these results, we conclude the following. (1) The transient picosecond resonance Raman spectrum of HbO_2 is essentially due to a reorganisation of the porphyrin core through a change in the electron distribution resulting from the departure of the oxygen ligand. (2) The time required for the reorganisation in the structure of the porphyrin core is less than 30 ps. (3) The picosecond time-resolved resonance Raman spectra of haemoglobin are related to the haem structural state with the iron atom pentacoordinated in a *R* protein conformation before its return to the *T* stable position. (4) The picosecond time-resolved Raman data show that the haem structure is at most only slightly dependent on the protein conformations, and that the porphyrin backbone structure is not very flexible. This conclusion agrees with X-ray experiments¹⁷.

Up to now, using CARS¹⁸ and the nanosecond time-resolved resonance Raman technique^{19–21}, no significant transient constraints have been observed between photodissociated HbCO and stable deoxyhaemoglobin. Because the change in haem geometry is not associated with the *R* or *T* globin state, it is conceivable, however, that the non-equilibrium globin structure brings about some reorientation between the haem and the globin and slight changes in the haem electronic repartition.

These relaxation processes must be more sensitive to electronic absorption spectra than to resonance Raman spectra. Lyons *et al.* could have seen this effect in the slight spectral shift of 3 cm^{-1} between unliganded haem from stable deoxy- and photodissociated haemoglobin²¹. Unfortunately, the digital technique with our dispersion is not refined enough to obtain a precision of better than 4 cm^{-1} on the absolute frequencies, and in the present work, we cannot draw clear conclusions on the existence of this small shift.

Using conventional continuous-wave resonance Raman spectroscopy, previous workers have already observed that the porphyrin spectra are not modified by the *R* and *T* shapes of apoprotein^{22,23} and that the haem structure (liganded or not) is independent of the apoprotein nature—amino acid composition, protomere associations, chemical modification²⁴. Nevertheless, with respect to normal haemoglobin, only very slight shifts in Raman frequencies have been reported for a modified haemoglobin²⁵. As the oxygen affinity of haemoglobin depends

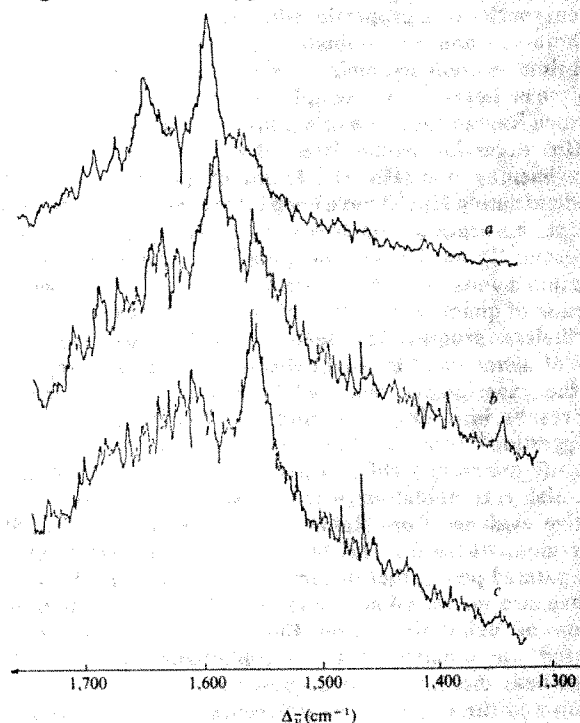


Fig. 2 Transient resonance Raman spectra. *a*, Fully oxygenated haemoglobin; excitation 15 ns, 15 laser pulses, 3 mJ per pulse. *b*, Partially photo-deligated haemoglobin; excitation 30 ps, 100 laser pulses, 1.2 mJ per pulse. *c*, Unliganded haemoglobin from stable deoxyhaemoglobin; excitation 15 ns, 20 laser pulses, 3 mJ per pulse. The same spectra were obtained from photo-deligated HbCO .

on the integrity of the protein structure^{26,27}, we suggest that the protein plays its part by acting directly on the charge of the iron atom and does not affect the geometric state of the haem. Furthermore, the protein relaxation process should be influenced by the structural and electronic state of the haem (including the iron ionic state, iron bound or not). This may explain some ambiguities and disagreements between recent electron paramagnetic resonance measurements²⁸, EXAFS data¹⁶ and resonance Raman work^{24,28,29}.

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Transient Raman study of CO-haemoprotein photolysis: origin of the quantum yield

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Carbon monoxide and haemoproteins (Fe^{2+}) form ligand-protein complexes that are photodissociated with high quantum efficiency by visible light^{1,2}. By photolysing the CO-protein complex with pulsed laser radiation it is possible to generate transient species with properties which reflect both the nature of the photolytic and recombination processes as well as non-equilibrium protein dynamics. Using a single pulse both to photolyse and examine the sample, we previously reported³ the resonance Raman spectrum of a transient species of haemoglobin (Hb) occurring within 10 ns of the photodissociation of carboxyhaemoglobin (HbCO). Transient species generated by photodissociating HbCO have been intensively studied by using transient absorption spectroscopy covering picosecond^{4–6}, nanosecond^{7–10} and longer time scales^{11–15}. The overlap of the absorption bands related to various species can hamper the extraction of quantitative information from absorption spectra. Nevertheless, progress has been reported, in particular, the results of nanosecond transient absorption studies^{9,10} reported after the experiments described here were well under way. Those results, including re-interpretation of earlier data⁸, led to the suggestion^{9,10} that photolysis of HbCO actually leads to a near-unity quantum yield of a deoxy-Hb species, followed by substantial recombination within 100 ns. We present here definitive evidence from Raman spectra of the 1,350–1,380- cm^{-1} region, where the HbCO and deoxy-Hb species exhibit well separated peaks, that this interpretation is indeed correct. We have also measured accurately the HbCO population as a function of delay time after full photolysis, and thereby measured the kinetics of the recombination process. Our findings bear directly on such questions as geminate recombination and the origin of the difference in quantum yield of photodissociation for HbCO and carboxymyoglobin (MbCO). These two systems exhibit very different quantum yields when strongly pumped in the visible: MbCO undergoes 97% photolysis whereas HbCO undergoes only 45–47% (ref. 15).

The apparatus used was similar to that reported previously³, except that the resonance Raman spectrum was generated by a 10-ns dye laser pulse (Moletron UV 24 and DL 14) fired with

an adjustable delay (up to 1 ms) after the pump pulse from the frequency doubled (5,320 Å, 20 mJ) output of a ND:YAG laser (Holobeam 500 QG). The spectra discussed below were obtained using between 4,100 Å and 4,350 Å (≤ 0.1 mJ) probe pulse excitations. As before³ the spectrum is accumulated by an optical multichannel analyser, based on an SEC Vidicon tube. The sample configuration has been changed to a flowing cell with the temperature and atmosphere of the sample under external control. A more detailed account of the apparatus is reported elsewhere¹⁶. Both Hb and Mb solutions (0.1 mM in haem, pH 7.5 phosphate buffer) were maintained at 10–20 °C under one atmosphere of CO. The solutions were initially degassed and treated with dithionite.

Spectra were recorded covering the region 1,225–1,575 cm^{-1} . The most prominent feature in this spectral region for Soret band excitations is the polarised peak which occurs at 1,357 cm^{-1} for deoxy-Hb, 1,373 cm^{-1} for HbCO and at 1,377 cm^{-1} for HbO₂ with similar values for Mb. The present study will deal only with the behaviour of this peak. A series of typical spectra for Hb and Mb is shown in Figs 1 and 2, respectively. Firing the dye laser before the photolytic pulse (a), we find that the spectrum reflects predominantly unphotolysed material (photodissociation by the probe pulse has been minimised by focusing the beam to a line). Within the first few nanoseconds after photolysis, the HbCO and MbCO peaks have completely disappeared and a peak in the region of the deoxy species has appeared (b). This observation is consistent with previous results obtained using a single pulse^{3,17,18} and using CARS¹⁹. At later times a difference is observed in the two systems. In the Hb system the HbCO peak regains nearly 50% of its intensity (Fig. 1c) within 100 ns of photolysis. On the same time scale (Fig. 2c) no corresponding change is observed in the Mb spectrum. At much later times the expected bimolecular recombination¹³ takes place in both Hb (Fig. 1d–g) and Mb (Fig. 2d–f). The transient deoxy peak is of better quality in the spectra excited with 4,200 Å and its position at early times (< 1 μs) is shifted slightly to lower energy than that associated with the steady-state deoxy-Hb (T) species. This shift is probably due to the effect of the structure of the haem pocket on the porphyrin^{20,22}.

At first glance, the intensity of the deoxy peak in the Hb system (Fig. 1) seems to be constant during the fast recombination process. This suggests that a species may exist after photolysis which is 'silent' in Raman scattering, and which subsequently decays into HbCO. To analyse this possibility more carefully, we used a nonlinear least-squares analysis to fit spectra similar to Fig. 1c–g, at 10 ± 2 °C, to a weighted average of

spectra similar to Fig. 1a, b.

$$S(\Delta\nu) = A_{\text{CO}}S_a(\Delta\nu) + A_{\text{deox}}S_b(\Delta\nu) + B$$

where $S_{a,b}(\Delta\nu)$ represent the corresponding points of the spectra in Fig. 1a, b. The three parameters A_{CO} , A_{deox} and B are varied to achieve a least-squares fit to $S(\Delta\nu)$ over the range shown. The results of this analysis are shown in Fig. 3, where we plot the weighting factors A_{CO} and A_{deox} against delay time. It is significant that, although all three parameters were varied independently, the value of B was consistently insignificant (<5% of the peak) and the sum of A_{CO} and A_{deox} was consistently between 95% and 110%. These results were so striking that we decided to reject the two points (out of 28) for which they did not apply. The quality of the fit was quite good for times below 400 ns. Data were taken at excitation frequencies from 4,350 Å, where the ratio of the deoxy-Hb Raman cross-section to that for HbCO is about 3.0, to 4,150 Å, where it is about 0.3. As shown in Fig. 3, the population values determined by our analysis, in the range studied, did not depend on excitation frequency outside an experimental error of about 10% for delays below 400 ns. Thus, we conclude that (1) for times between 10 and 400 ns, the Raman enhancement does not change appreciably for either species, nor do their respective line shapes; (2) for each ligated haem which appears, an unligated haem disappears; (3) 41% of the photolysed haem groups initially observed at 15 ns after photolysis recombine within 200 ns. A fit to the points under these assumptions, shown in Fig. 3, gives a recombination lifetime of 65 ns for the fast process. The occurrence of such a fast recombination process has also been inferred^{9,10} from transient absorption studies. Note that the absorption lines of the two species are not completely resolved spectrally.

The analysis discussed above was only qualitatively useful beyond 400 ns, because the position of the peak for the deoxy-Hb species begins to shift noticeably at that delay. The deoxy peak moves 3–4 cm^{-1} , to higher energy, between 0.5 and 4 μs delay. The resonant enhancement also evidently increases. (The amount of this increase is a function of excitation frequency, as expected. At 4,200 Å the increase is about 10%). These changes, probably the spectral signature of the initial events

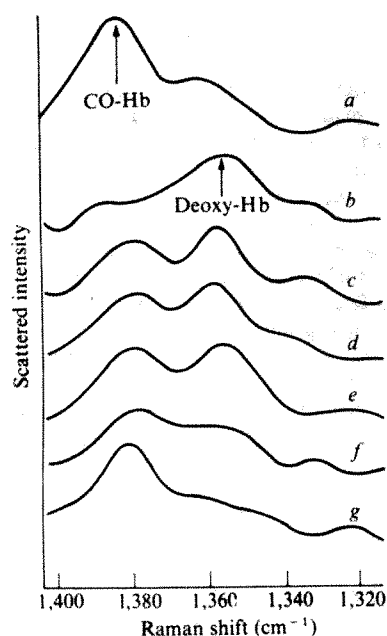


Fig. 1 Raman spectra of HbCO taken at various delays after photolysis with a time resolution of 10 ns. The various traces, shown displaced vertically for clarity, correspond to: a, before photolysis; b, 15 ns after; c, 100 ns; d, 1 μs ; e, 10 μs ; f, 100 μs ; g, 1 ms. The small shifted 'CO' peak in trace b may be due to the presence of a small amount of HbO_2 . Note the reappearance of HbCO in trace c.

heralding the R - T transition in the partially ligated Hb^{22} , caused spurious changes in the population values extracted in the least-squares analysis, and the resulting fits were noticeably poorer. Hence, the results for these later times could not be treated as quantitatively as those displayed in Fig. 3. Nevertheless, the results at early times yield striking evidence that the quantum yield for photolysis of HbCO deviates from 100% due to a recombination of photolysed haems, with a characteristic time of 65 ± 25 ns. Correcting our results for the recombination taking place in the first 15 ns, we obtain an estimated quantum yield of $54 \pm 4\%$ (that is, 46% recombination total). Comparing this with the reported value of the quantum yield of 47% (53% unphotolysed) measured at longer times, we see that the absolute quantum yield for the primary photolysis process (that is, the Fe-CO bond cleavage) is about 90%, a value similar to the total yield observed in MbCO, where the fast recombination does not occur.

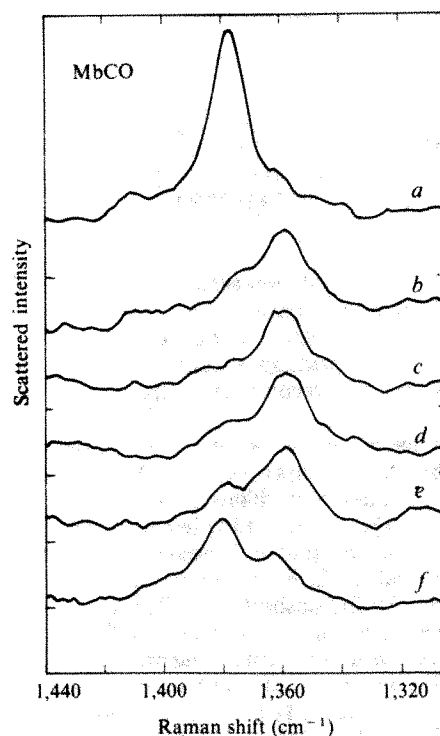


Fig. 2 Transient Raman spectra of MbCO analogous to Fig. 1. The traces represent: a, before photolysis; b, 15 ns after; c, 100 ns; d, 1 μs ; e, 10 μs ; f, 1 ms. No significant recombination is observed for times < 100 μs .

The observation of the nanosecond recombination in Hb but not in Mb and the known similarity in electronic properties of the haems in both proteins tend to rule out explanations for the occurrence of this sizeable fast recombination based solely on the electronic properties of the iron porphyrin. On the other hand, there are germane properties of haemoproteins, such as the quantum yield for the photodissociation of the CO-haem complex, which are dependent not only on the specific protein but also on the quaternary structure of a given haem protein¹⁵. Consequently, it seems highly plausible that variation in protein structure about the haem rather than major variations in the electronic state determinants of the photolytic pathway is responsible for the major differences²¹ in the behaviour of photolysed carboxyhaemoproteins.

The rapidity of this process suggests that the recombination process involves photodissociated CO molecules that have remained in the vicinity of the haems, that is, geminate recombination. This interpretation is strongly supported by our observation that the fast recombination is insensitive to the CO concentration (0.02–2 atm). This lack of sensitivity to the CO

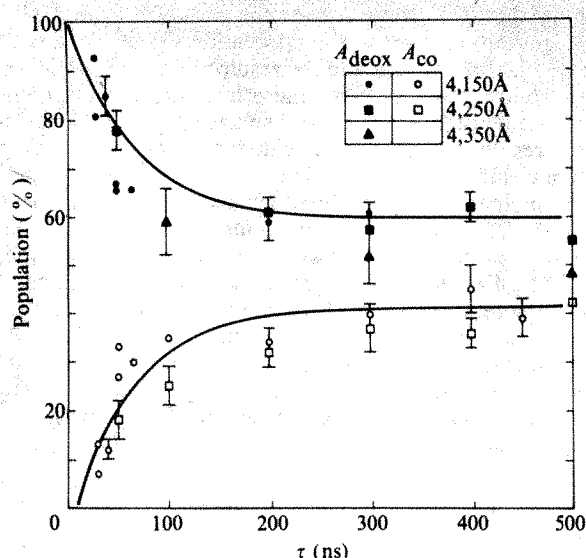


Fig. 3 Population weight factors, A_{deox} , A_{CO} , resulting from the least-squares analysis described in the text. The wavelengths in the key denote the dye laser excitation used to obtain the resonance Raman spectra.

concentration was also observed in transient absorption measurements at discrete frequencies¹⁰. In the absorption study it was also found⁹ that the amount of recombination decreased with increasing temperatures. From these dependencies we conclude that the fast recombination is, in fact, geminate in origin.

The deoxy-haem species appears in transient absorption spectra within picoseconds of photolysis⁴⁻⁶. Hence, any geminate recombination mechanism must include some means of trapping the CO molecule in the haem pocket after cleavage of the Fe-CO bond for a time τ of about 65 ns. Furthermore, this trapping, if it involves the Fe ion, must occur in a way that does not produce the HbCO absorption or Raman spectra. It seems likely, then, that the trapping is related to direct interaction of the CO molecule with the surrounding protein. This interaction probably takes the form of CO-binding sites (or potential minima) on the protein. This direct CO-protein interaction, responsible indirectly for the geminate recombination, would explain the protein and conformation dependence of the quantum yield.

The difference between the MbCO and HbCO systems could lie either in the dynamics of escape and recombination from similar sites, or in the existence of different kinds of sites. In either case, the α - and β -chains would be expected to exhibit differences in their behaviour. The conformation dependence of the quantum yield¹⁵ may also be explained on this basis. Furthermore, the proximity of the geminate recombination fraction to 50% in the R conformation strongly suggests that one chain predominantly undergoes recombination and the other does not. Indeed, experiments with isolated chains do indicate differences¹² at 290 K ($QY_{\beta} \approx 8\%$, $QY_{\alpha} \approx 90\%$). However, because this relationship is strongly temperature dependent, it may not hold at physiological temperatures, where the quantum yield for HbCO is much higher, and the geminate recombination is correspondingly reduced.

Thus, we have shown that transient resonant Raman spectra of photodissociated HbCO support the basic features of the suggestions made recently on the basis of transient absorption experiments^{9,10}. The better separation of the transient species in the Raman spectra has made possible a more complete determination of the populations as a function of time after photolysis. Our findings indicate that differences in the quantum yield for photodissociation of carboxyhaemoproteins can originate from differences in geminate recombination rates.

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Correlation of IR spectroscopic, heat capacity, diamagnetic susceptibility and enzymatic measurements on lysozyme powder

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The interaction between protein and water is of fundamental importance for processes ranging from protein folding and enzymatic activity¹ to anhydrobiosis². In this letter we bring together results from diverse types of measurements to give a unified picture of the hydration process for lysozyme. The data come principally from experiments with protein films and powders. The principal aim is to examine the relationship between the sites of water interaction, the extent of coverage, and the enzymatic activity, thus providing a better understanding of the relationship between water and enzyme dynamics³.

Figure 1 describes the results of measurements on the heat capacity⁴, enzymatic activity⁵, IR spectroscopic properties⁶, and diamagnetic susceptibility⁷ of lysozyme powders as a function of hydration level (h , g of water per g of protein).

The appearance of a positive peak at $1,580 \text{ cm}^{-1}$ in the IR difference spectrum (Fig. 1a) indicates the formation of carboxylate species⁶. Apparently, drying the protein below $0.05h$ has produced inversion of the pK order for carboxylic and basic groups, resulting in charge neutralisation by proton transfer. This implies that interaction with water is associated with proton redistribution—that is, normalisation of the pK order with deprotonation of carboxylic acid groups and protonation of basic groups, presumably amino groups, to give the ionisation state found for the protein in solution. A proton redistribution process is expected to produce a rise and fall in the heat capacity, as is observed at $0.05h$ (Fig. 1d).

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The discontinuity in several properties at 0.07h must represent at least changes in water-water and water-protein interactions, because there are effects on the IR spectroscopic properties of both water (Fig. 1c) and protein (Fig. 1a, b). The change in slope of the heat capacity function at 0.07h suggests that the water-protein arrangements have greater freedom above the discontinuity. In this regard, the partial specific heat capacity of the water bound below 0.07h is similar to that of ice or water vapour, while that above 0.07h is greater than that of liquid water, which has twice the specific heat capacity of ice. It is important that the fine structure of the amide I' band is unchanged above 0.07h, and thus significant contributions to the heat capacity from changes in protein conformation can be ruled out⁶.

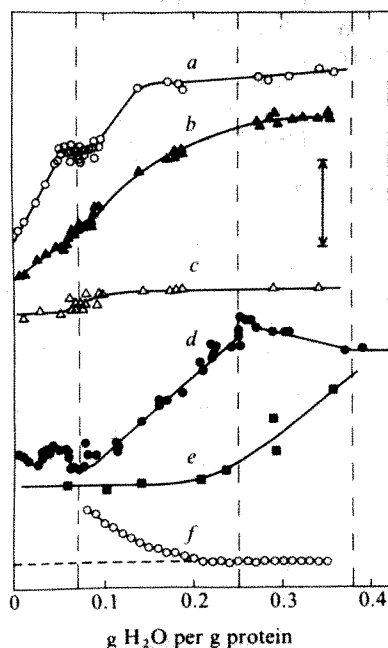


Fig. 1 Properties of the lysozyme-water system as a function of water content. The correspondence between the vertical bar in the figure and the units of measurement is given separately for each curve. From top to bottom the curves are: *a*, Absorbance at the carboxylate band maximum ($1,580\text{ cm}^{-1}$), measured at 38°C ; units, 0.35 A. *b*, Change in the amide I' band, measured at 38°C as the difference between the negative ($1,690\text{ cm}^{-1}$) and positive ($1,645\text{ cm}^{-1}$) extrema about the isosbestic point of the differential spectrum; units, 0.17 A. *c*, Frequency of the highest intensity maximum of the OD stretching band of adsorbed D_2O , measured at 38°C . The total shift is from $2,550\text{ cm}^{-1}$ to $2,580\text{ cm}^{-1}$. *d*, Apparent specific heat capacity of lysozyme, for 25°C ; units, $0.2\text{ J K}^{-1}\text{ g}^{-1}$. This function is a measure of the excess heat capacity of the system per g protein. *e*, Enzymatic activity, measured at 25°C ; units, $5 \times 10^{-6}\text{ s}^{-1}$. *f*, Diamagnetic susceptibility, measured at 25°C and expressed as differential susceptibility per g of water adsorbed; units, $0.55 \times 10^{-6}\text{ e.m.u. g}^{-1}$. The high hydration limit is equal to the value for liquid water ($0.721 \times 10^{-6}\text{ e.m.u. g}^{-1}$).

The continuous changes in heat capacity and IR spectroscopic properties from 0.1 to 0.2h represent binding of water at amide and carboxylate sites and presumably also other charged or polar sites. Saturation of these sites, if it is complete before significant covering of nonpolar elements, is expected to require^{8,9} about half the water needed for monolayer coverage, as is observed (0.2 versus 0.38h). The polar atoms are largely in the backbone and side-chain amide groups. The change in the amide I' band is a local effect produced by association of the amide group with water and not the result of changes in protein conformation¹⁰.

At 0.2–0.25h the carboxylate and carbonyl sites are saturated (Fig. 1a, b). The full coverage of the hydrogen-bonding sites is reflected in the differential diamagnetic susceptibility, which at this hydration level reaches the value for liquid water. At 0.25h the heat capacity rises (Fig. 1d) and then falls to the dilute solution value at 0.38h. This behaviour must be associated with coverage of the nonpolar elements of the protein surface, in view of the IR spectroscopic results that show saturation of hydrogen-bonding sites at lower hydration. The rise and fall in the heat capacity can be understood as a transition associated with covering of the least-strongly interacting regions of the surface. Statistical mechanical arguments indicate that a transition is expected at high coverage for adsorption of water on a heterogeneous surface¹¹. The magnitude of the 0.25h heat effect indicates that as many as 100 water molecules could be involved⁴.

The appearance of enzymatic activity at 0.2–0.25h (Fig. 1e) coincides with the saturation of the hydrogen-bonding sites and with the beginning of the coverage of the nonpolar regions of the surface. The catalytic activity is not simply reflecting water as a substrate in the hydrolytic reaction, because the dependence of the reaction velocity on water activity is tenth order. The new water arrangements on the protein surface that obtain above 0.25h may be required for catalysis. Spin-label studies⁵ show that these have greater motional freedom.

The apparent specific heat capacity shows no change between 0.38h and dilute solution. Because the heat capacity reflects all equilibria of non-zero enthalpy as well as the heat capacities of the components of the system, we regard this as sufficient evidence for the statement that the changes in thermodynamic properties associated with protein hydration are complete both for the protein and the solvent at 0.38h. This amount of water is barely sufficient to constitute monolayer coverage⁴ and corresponds to 300 molecules of water per protein molecule. Higher hydration is required to establish fully the kinetic properties for the dilute solution state.

We infer that between 0.1h and dilute solution, there can be no substantial change in protein structure. This is concluded from the monotonic behaviour of the IR spectroscopic properties of the amide and carboxylate groups between 0.07 and 0.25h, from the monotonic rise of the enzymatic activity observed above 0.2h, and from the constant apparent specific heat capacity observed from 0.38h to higher levels of hydration.

The following picture of the hydration process emerges from the findings and comments given above. The first water bound interacts with ionisable groups to produce proton redistribution. There is a clear transition in the hydrogen bonding between water and protein and water and water at 0.07h. This probably represents a change in the water molecule distribution about the protein surface, followed by an increase in freedom of the water-protein system. At 0.2–0.25h there is a major event in the hydration process, which is seen most clearly in the heat capacity and which coincides with the onset of enzymatic activity and the reaching of the final value of the response of the system to a magnetic field. Completion of monolayer coverage is at 0.38h, a value substantially greater than that for first observation of enzymatic activity. The protein with monolayer hydration shell must mesh simply with the bulk solvent.

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MATTERS ARISING

Age and significance of alluvium in the Windrush Valley

RECENTLY Hazelden and Jarvis¹ reported a radiocarbon date of $2,660 \pm 85$ yr BP for *in situ* roots in the gravel underlying the alluvium of the River Windrush, Oxfordshire. They suggest that this date represents the regional onset of clay deposition following forest clearance. This hypothesis discounts the possible role of channel migration which, although mentioned by the authors, was not discussed further.

Many rivers in southern and eastern England have gravel underlying the floodplain. Such rivers include the Trent, Nene, Great Ouse and Thames, together with many of their tributaries. The underlying gravels are continuous with those gravels lying about 1 m above the floodplain, forming the first or floodplain terrace. The majority of these first-terrace gravels seem to be middle-late Devensian in age², and they represent a phase of aggradation probably caused by increased sediment load during periglacial conditions. As sediment supply decreased, when periglacial conditions ended, downcutting occurred, giving rise to the wide channel now infilled by alluvium.

Deposition of fine-grained alluvium would be expected to have started as the ameliorating climate led to the formation of meandering rather than braided rivers. Dates from the base of the alluvium are highly variable, ranging from 10,000 to 2,500 yr BP (refs 3,4) and probably reflect the time at which the floodplain deposits were last reworked by a migrating meander rather than an ubiquitous change in conditions.

The deposits of the River Windrush^{1,5} are very similar to those found elsewhere and it therefore seems entirely plausible that an alder tree, growing through a pre-existing floodplain with roots in the underlying gravel, should have been eroded by a migrating meander at 2,660 yr BP with no change in sediment supply, climate or base level.

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HAZELDEN AND JARVIS REPLY—While this is a possible alternative explanation, it seems to us a less likely one. The truncation of the alder roots is associated with the gravel/clay interface; nowhere in the area have we seen the roots in the clay. Buried A horizons are common within the clay alluvium, and the sequence of deposits is the same over a large area of the floodplain, so it seems that extensive reworking by migrating meanders has not taken place. In view of this and the corroborative evidence from Farmoor we think our explanation more likely.

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Trace metals in remote Arctic snows: natural or anthropogenic?

NRIAGU¹ states that "It is encouraging that the ratios of the 1901–10 to the 1971–80 emissions of metals calculated from the data in Table 3 are consistent with the historical changes in rates of trace metal deposition observed in polar ice cores in the Northern Hemisphere". Some of the data cited^{2–4} in support cannot now be considered reliable, and we believe that the remaining data do not support his statement. The historical changes observed in polar ice cores appear much smaller than corresponding changes in worldwide anthropogenic emissions presented by Nriagu in Table 3.

The rate of trace metal deposition is a complex function of many variables, predominantly that of the deposition rate of snow. Reported deposition rates have been calculated from observed metal concentrations and from accumulation rates of snow which have not apparently

changed significantly during the period in question. There is no established theoretical or empirical relationship linking concentrations of metals in precipitation to those in air⁵, but most workers have assumed that the relative abundance of metals in snow reflects the composition of aerosols. Whilst this has been substantiated at the South Pole^{6,7}, recent work has revealed considerable differences in composition between Arctic aerosol and snow at Barrow, Alaska⁸.

Of the data cited by Nriagu, results obtained from a temperate glacier in Norway⁴ contain insufficient information about the local geology and analytical procedures, so that the abnormally high metal concentrations quoted must be treated with caution. The Dye-3 data² for recent snow are now believed^{3,9} to reflect local contamination from the nearby base manned since 1959. Using the data from Camp Century² and Station Milcent³, the ratios of metal concentrations in snow deposited during the periods pre-1900 and post-1960 have been calculated. These values, together with their standard deviations, are given in Table 1. Average concentrations in snow rather than ranges have been considered since the spread of extreme values may in part arise from seasonal variations¹⁰ and maximum values often represent contamination. The resulting ranges are so wide that no meaningful conclusion can be made.

Table 1 shows that the concentration ratios in snow are considerably smaller than the corresponding anthropogenic emission ratios. These discrepancies can be partially explained if the natural emissions quoted as most acceptable by Nriagu are added to the anthropogenic emission figures (last column of Table 1). There is even closer agreement if higher natural emission values (within the limits of values given by Nriagu and others¹¹) are chosen. It is to be expected that a higher proportion of trace elements of natural origin (particularly volcanogenic) will be transported long distances compared with emissions of anthropogenic origin, a major proportion of which return to Earth

Table 1 Comparison of global metal emission data with metal concentrations in Greenland snow

Metal	Anthropogenic emission ratio (from Nriagu's Table 3)	Camp Century snow concentration ratios ^{2*}	Station Milcent snow concentration ratios ^{3*}	Emission ratio including natural sources ¹
	1971–80	Post-1960	Post-1960	1971–80
	1901–10	Pre-1900 $\pm \sigma$	Pre-1900 $\pm \sigma$	1901–10
Cd	8.3	0.25 ± 0.27	1.0 ± 0.5	4.8
Cu	11.0	2.1 ± 1.6	—	3.2
Pb	9.1	—	3.2 ± 1.8	6.3
Zn	8.3	—	2.9 ± 1.65	4.5

* From averages of metal concentrations in snows.

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close to their source¹². Volcanism has been invoked to account for the particularly high proportion of natural emissions in Antarctic snow⁶.

A substantial natural input of trace metals to Greenland snow has been reported by other workers¹³, and a large enrichment of heavy metals in comparison with crustal reference elements has been firmly demonstrated in both ancient and modern polar snows^{13,14}. Until the mechanisms of trace metal deposition are clearly understood, any attempts to assess temporal changes in atmospheric loadings from evidence in ice cores must be tentative.

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NRIAGU REPLIES—Landy *et al.* have misinterpreted my report¹. I was careful to say changes in rates of trace metal deposition rather than the ratios in trace metal concentrations in the ice layers. My statement clearly pertains to dF/dt (where F is metal flux rate at each ice layer and t is time), and is quite different from the linear relation, C_1/C_2 (C_1 , C_2 are metal concentrations of dated ice layers) assumed by Landy *et al.* The data tabulated by Landy *et al.* may thus bear no relationship whatsoever to the statement in my paper.

Landy *et al.* have dismissed the data of Jaworowski *et al.*² because “it contained

insufficient information about local geology and analytical procedures”. However, more than a page of this paper was devoted to materials and methods and included the cogent reference to the work on the characteristics of the Storebreen glacier. The high values of metals reported may not be due to sample contamination as such but may instead be related to deposition of long-range transported material from industrial centres of Europe (such as sulphate deposition and acid rains, see refs 3 and 4). In this sense, the Storebreen glacier probably contains a better record of changes in intensities of metal emissions in Europe.

The use of average concentrations of metals in ice layers deposited over a period of several decades or centuries (before 1900) in deriving the data listed by Landy *et al.* is misleading. It assumes that the rate of metal deposition was constant before AD 1900, which is contrary to the available evidence^{5,6}. I have calculated the ranges in the post-1960 to 1800–1900 ratios of reported trace metal concentrations in the ice fields of the Northern Hemisphere (Table A). The maximum and minimum values in reported metal concentrations during 1800–1900 and since 1960 have been used to derive the data listed. Obviously the ranges in the ratios are quite wide and the ratios obtained using my emission data are either within or fall near these ranges. This is ‘encouraging’ considering that the emission data are only order of magnitude estimates anyway.

The contention by Landy *et al.* that the trace metals in Arctic snow are derived mainly from natural sources is probably not valid. Rahn and his colleagues^{11,12} in fact have recently presented rather convincing evidence that the aerosols at widely dispersed Arctic sites of northern Norway, northern Greenland and Barrow, Alaska are strongly pollution-derived, particularly during the winter time. Before that, Chow and Earl¹³ used the isotope ratio method to show that most of the lead in Greenland glacier is of anthropogenic origin. Ancillary evidence in support of the anthropogenic origin of lead deposited in the snow fields of Northern Hemisphere are given by Settle and Patterson¹⁴. Note that the ratios of the present day deposition rates for Pb, Cd, Cu and Zn at Centrale, Greenland and at Dome C, Antarctica are

65, 16, 16 and 91 respectively¹⁵; the ratios of the Pb, Cd, Cu and Zn concentrations in present day surface snow at the two sites are 6.9, 1.6, 1.6 and 9.4 respectively¹⁵. Presumably, the much higher concentrations and deposition rates at the Arctic site vis-a-vis the Antarctic location is related to the fact that most of the anthropogenic emissions of metals occur in the Northern Hemisphere.

It should be emphasised, however, that thorough studies are needed on the deposition mechanisms and on the factors controlling the elemental composition of polar snow and ice before the metal concentrations in the ice layers can be related quantitatively to the atmospheric metal burdens. On this point, I completely agree with Landy *et al.*

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BOUSTRON COMMENTS—With regard to the above exchange of views I would like to comment on the available polar ice and snow historical records of the variations of the atmospheric concentrations of the five metals (Pb, Cd, Cu, Zn and Ni) discussed in Nriagu's paper¹, in the remote areas of the Northern Hemisphere during the past few centuries. Data such as those published for a temperate glacier in southern Norway² and quoted by Nriagu are not polar data, so that the only genuine polar historical records presently available for the Northern Hemisphere are those obtained in Greenland. Numerous data have been published on the trace metal content of the successive snow and ice layers deposited in the Greenland ice cap during the past few centuries. Unfortunately, many of these data are probably unreliable because they were plagued by severe contamination problems during field collection or (and) during laboratory analysis. It is very important then to estimate which of these data, if any, are reliable: this can be looked at by a detailed examination of the collection and analytical procedures described by the authors, with special reference to the use of high performance clean rooms and to

Table A Ranges in the post-1960 to 1800–1900 ratios of metal concentrations in the Arctic snow fields

Site	Cd	Cu	Pb	Zn
Milcent ⁷	1–2	—	1–7	1–8
Milcent ^{7,8}	<1–10	—	3–20	1–20
Dye-3 ⁹	—	—	9	—
Dye-3 ¹⁰	10–600	1–100	—	<1–40
Camp Century ¹⁰	<1	1–7	—	—
Camp Century ⁶	—	—	<1–>12	—
Jotunheimen Mountains ²	<1–68	—	<1–6	—

blank estimates both for field sampling and laboratory analysis.

Concerning the five metals discussed in Nriagu's paper, reasonably reliable Greenland data seem to be available only on the variations of the concentrations of Pb, Cd and Zn during the past 800 yr (refs 3, 4). On the other hand at present there seems to be no reliable Greenland data on the past variations of the concentrations of Cu and Ni. The reliable Pb, Cd and Zn data are shown in Fig. 1 for the past 300 yr; they do not include the Cd and Zn data published by Weiss *et al.*⁵, which have been shown recently^{4,6} to be erroneously high (by up to 2–3 orders of magnitude for Cd) probably because of severe contamination problems.

The interpretation of the variation profiles shown in Fig. 1 to assess man's global impact on the low atmosphere in the remote areas of the Northern Hemisphere is, however, still questionable for various reasons: (1) the scarce data available do not provide continuous variation profiles; (2) they have been obtained in several widely separated geographic sites; (3) some of the concentrations measured in the recent surface layers (post-1960 data) could be erroneously high because of local contamination problems in the vicinity of the Greenland stations; (4) there are still doubts on the efficiency of the decontamination procedures⁴ used to clean the deep ice core samples whose analysis has produced most of the pre-1900 available data; (5) for the years 1880–1960, which are the most interesting to assess man's impact, there are no Cd and Zn data, and only few Pb data; (6) the variation profiles could have been influenced by the arrival of volcanic aerosols emitted by the eruptions of the near Iceland volcanoes or by major eruptions which occurred in the world, such as Agung eruption (1963)^{7,8}; (7) the impurity content of Greenland snows could be influenced strongly by local crustal sources associated with the large ice free coastal areas of Greenland⁶; (8) there is no clear demonstration available that there are no chemical-fractionation processes at the air-snow interface in Greenland, such as the ones documented recently in Alaska⁹, which could lead to misleading interpretations of the snow and ice records in terms of atmospheric records.

Figure 1 suggests, however, that there has been no definite increase trend for Cd and Zn concentrations during the past 300 years. This is also the case for Pb before approximately 1940; after this date, a significant but limited increase of the concentrations of Pb seems to have occurred (about threefold increase). From the available data it seems, therefore, that the concentrations of Pb, Cd and Zn in Greenland snow and ice have not increased markedly during the past few centuries except probably for Pb since 1940, then suggesting that there has been no significant increase of the atmospheric

concentrations and then of the fallout fluxes F of these metals in the remote areas of the Northern Hemisphere since the beginning of the industrial revolution, except probably for Pb after 1940: the changes in anthropogenic emissions of these trace metals calculated by Nriagu¹ (from 1850–1900 to 1971–80: 19.5-fold increase for Pb, Cd and Zn) are then clearly much higher than the very limited historical changes in the fallout fluxes F of these metals observed in the remote polar areas of the Northern Hemisphere. (At a given polar site, the tropospheric fallout flux F of a metal during a given year is

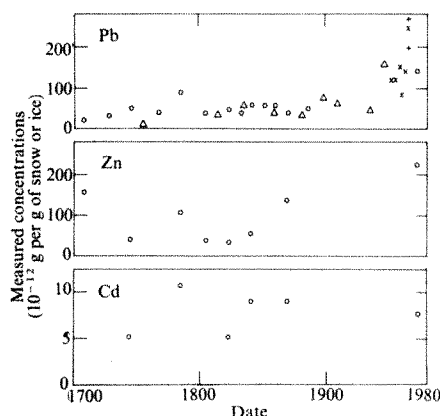


Fig. 1 Greenland: available reasonably reliable data on the concentrations of Pb, Zn and Cd in the successive snow and ice layers deposited since 1700. The different symbols correspond to separate sampling locations (O, Milcent; Δ , Camp Century; +, 45 km ESE from Camp Century; x, 80 km ESE from Camp Century).

calculated by glaciologists as $F = CA$ where C refers to the mean measured concentration of the considered metal in the snow or ice layer deposited during this year and A is the annual snow accumulation during this year. In Greenland, A has been shown not to have changed markedly during the last centuries¹⁰. Note that at present it seems impossible to derive from the scarce Greenland data shown in Fig. 1 any useful estimate of the time derivative dF/dt to which Nriagu claims to refer in his reply, and then to check whether the historical changes of dF/dt support the changes in anthropogenic emissions he calculated or not.

The available snow and ice Greenland data suggest strongly that the influence of human activity on the trace metals content of Greenland snow and ice, and therefore probably on the trace metals content of the low atmosphere in the remote areas of the Northern Hemisphere, is actually negligible for Cd and Zn and makes only a limited contribution for Pb: the high atmospheric enrichments observed presently for these metals in various remote areas in the Northern

Hemisphere^{11,12} are then very likely linked with natural processes such as volcanism.

However, it would be highly desirable to confirm and to complete the scarce Greenland data, through a cooperative analysis of new samples to be collected for this purpose, especially to get detailed continuous data on the trace metals content of the snow and ice layers deposited in Greenland during the past 100 yr.

Finally, I would like to comment on another point: the comparison¹¹ of the present day Pb, Cd, Zn and Cu concentrations measured in surface Greenland snows at Centrale⁶ with those measured in surface Antarctic snows at Dome C¹³. Present-day Greenland concentrations are higher than Antarctic concentrations probably principally because of the very different geographical locations and meteorological regimes of Greenland and Antarctica, and not only because of the fact that more than 90% of anthropogenic aerosols are emitted in the Northern Hemisphere¹⁴ as stated by Nriagu: Antarctica is indeed much more remote from any aerosol sources than Greenland, which is located near the North American continent, and is moreover protected from the influence of mid-latitude aerosol sources by the 50°S polar convergence.

From the present day surface concentrations at Dome C and at Centrale, and from the annual snow accumulation at these two sites, it is possible to get estimates of the present day fallout fluxes of Pb, Cd, Zn and Cu (ref. 11) in the central areas of Antarctica and Greenland: the fallout fluxes calculated at Centrale, Greenland are much higher than those obtained at Dome C, Antarctica, as pointed out by Nriagu. But this strong difference is certainly mainly related to the fact that the annual snow accumulation at Centrale ($34.3 \text{ g H}_2\text{O cm}^{-2} \text{ yr}^{-1}$) is one order of magnitude higher than the annual snow accumulation at Dome C ($3.5 \text{ g H}_2\text{O cm}^{-2} \text{ yr}^{-1}$).

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Polydipsia after intracranial injections—a property of NGF or a contaminant?

INTRACRANIAL injections of 2.5S NGF in adult rats have been shown to induce intense polydipsia¹. Subsequent studies from other authors confirmed this finding and, in addition, gave evidence of increased appetite for sodium solutions². The authors of this last article call attention on the similarity between these effects and those elicited by intracerebral renin injections. Previous work from this laboratory in collaboration with the Department of Pharmacology, University of Heidelberg³, showed that isorenin activity is present in 2.5S NGF preparations and is separated only after laborious additional purification steps. Thus, NGF prepared with the same method as that used for studies on induced polydipsia and sodium appetite still showed considerable amounts of renin-like activity. According to Cozzari *et al.*³ it was necessary to perform three further carboxymethyl-cellulose chromatographies after the usual procedure to prepare 2.5S NGF, in order to eliminate all renin-like activity previously detectable.

In view of the many effects which have been recently claimed to be caused by NGF intracranial injections^{4,5}, it seems essential that whenever high doses of NGF are needed to produce a given effect, it should be clearly established that the preparations are entirely free of contaminants. This precaution is of particular importance since NGF has been found to be tightly bound in mouse salivary extracts with other proteins endowed with enzymatic or other biological activities.

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LEWIS ET AL. REPLY—Levi-Montalcini is right to draw attention to the possibility that the effects of NGF on the central nervous system may be mediated by portions of the NGF complex other than the β subunit. As we pointed out in our report¹, the marked similarity between the effects of intracranial injections of 2.5S NGF and renin on thirst and sodium appetite suggested that the effects of 2.5S NGF might be mediated by the cerebral isorenin-angiotensin system. Activation of this system is an exceedingly potent stimulus to thirst² and sodium appetite³. We have since established that

7S NGF⁴ has similar effects on thirst and sodium appetite, and that both 2.5S⁵ and 7S NGF produced renin-like pressor responses when injected intravenously in intact and nephrectomised rats⁶. The doses of these NGF preparations needed to produce pressor responses are comparable to those used in the standard *in vitro* NGF bioassay⁷ and considerably less than the doses of NGF reported to induce growth in the sympathetic ganglia of newborn mice⁸. The pressor responses to 7S and 2.5S NGF were abolished by the angiotensin-converting enzyme inhibitor SQ 14,225, the angiotensin-receptor blocker saralasin, and NGF antiserum⁶. The same procedure also blocked the effects of these NGF preparations on thirst and sodium appetite⁹. Immunogenically pure β -NGF subunit¹⁰ from the mouse submandibular gland was devoid of pressor activity and had a much smaller and more variable effect on thirst and sodium appetite than other preparations of NGF⁶.

Our demonstration that NGF-induced thirst and sodium appetite are mediated through the formation of angiotensin II (AII) raises the possibility that other effects of NGF preparations are similarly mediated. For example, we have recently found that intracranial administration of renin, like 2.5S^{11,12} and 7S NGF, results in a marked increase in the activity of ornithine decarboxylase (ODC) in brain and liver⁶. These increases in ODC activity in response to renin and NGF are blocked by pretreatment with SQ 14,225 and are therefore dependent on the formation of AII⁶. The AII-dependent induction of ODC in the liver following NGF administration is due to activation of the pituitary-adrenal axis, as the induction is blocked by either hypophysectomy or adrenalectomy¹². Otten *et al.*¹³ recently reported that systemic injection of 2.5S NGF, the purity of which was controlled by SDS-polyacrylamide gel electrophoresis¹⁴, caused a rapid increase in plasma ACTH and corticosterone levels. However, systemic or central administration of AII also causes an increase in plasma ACTH and corticosteroid levels^{15–17}. Therefore, activation of the pituitary-adrenal axis following NGF administration may be due to the presence of a renin-like enzyme in the NGF.

Our findings complement those of Cozzari *et al.*¹⁸, who reported that NGF preparations from mouse submandibular gland are associated with a renin-like enzyme that can only be removed after much additional purification. Whether this renin-like enzyme or other enzymes^{19,20} should be regarded as integral components of mouse submandibular gland NGF complexes has not been resolved. However, unless renin-free preparations of NGF are used, the use of angiotensin antagonists such as SQ 14,225 and saralasin should be considered

obligatory, particularly when a biological effect attributed to NGF is known to be produced by renin or AII.

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Evolution by gene duplication in insecticide-resistant *Myzus persicae*

THE simple model of “a succession of tandem duplications of the structural gene”, proposed by Devonshire and Sawicki¹, cannot account as it stands for the apparent regularity of the variation in enzymatic activity of the organophosphorus (Op) insecticide resistance associated esterase (E4¹ or RAE²) observed in specimens of the peach potato aphid, *Myzus persicae*, from different laboratory stock clones, because the esterase does not have the same electrophoretic mobility or phenotype in all the clones compared.

The enzyme from the predominant Op resistant clone in British field populations³ (E4(MS1G) or RAE(+)²) stains after electrophoresis in two close but distinct bands, the rear band being fainter and staining more slowly². Aphids with this electrophoretic phenotype, but with higher enzymatic activity on the gel, have also been collected in the field², and these could have arisen by duplication of the E4(MS1G) gene, although other explanations are also possible, for example, that they are homozygous for E4(MS1G), or that they are a mutant in which E4(MS1G) has become more resistant to denaturation in gel assay

conditions. But the high activity enzyme in the laboratory clone French R and the very high activity enzyme in the highly Op-resistant glasshouse clone PirR (electrophoretically identical to that in the highly resistant clones from northern England and western Scotland⁴) both stain after electrophoresis in a single band with an electrophoretic mobility slightly but distinctly different from E4(MS1G) and from each other.

Because of this electrophoretic distinctiveness it is unlikely that either of these high activity mutant esterases could have arisen by a duplication of the E4(MS1G) gene, or that the higher activity esterase (E4(PirR)) could have arisen by a duplication of the gene for the lower (E4(French R)). A more reasonable explanation would be that E4(MS1G), E4(French R) and E4(PirR) arose independently in separate mutations in different Op-susceptible aphids.

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DEVONSHIRE REPLIES—The simple model of “a succession of tandem duplications of the structural gene” is based on the measurement of insecticide hydrolysis¹, and not simply on the subjective assessment of electrophoresis gels stained for naphthyl acetate-hydrolysing enzymes. The enzyme, E4, has been shown to be identical in susceptible and resistant aphids, and differences in activity are a direct consequence of the production of more of the same enzyme¹.

Esterase E5 (which probably corresponds to ‘the satellite’² to E4) is present in MS1G and French R, but not in the other five clones examined¹ and seems to occur only in untranslocated variants. Any association of this band with E4 is speculative, and our studies have shown that it plays no part in insecticide hydrolysis. Baker suggests that the six specimens from two sites, in which “the electrophoretic band (E4) appeared³ to be slightly retarded in mobility and without a satellite (E5?)” are associated with glasshouses. We have never detected such decreased mobility of E4 in slightly resistant variants among the several thousand insects examined from the field and glasshouses throughout the UK. The biochemical and toxicological significance of this putative electromorph has not been investigated, and it has little bearing on our conclusions. Although very resistant aphids (with 32 or 64 times as much enzyme) appear to have E4 with slightly lower mobility, on dilution the enzyme reverts to the characteristic mobility either when run alone or when coelec-

trophoresed with E4 from susceptible insects. It is therefore an artefact of the electrophoresis probably arising from the larger amount of protein present.

In view of the convincing biochemical evidence, and lack of ‘electrophoretic distinctiveness’ of E4 in the seven variants examined, we believe gene duplication to be the most likely cause of the overproduction of E4 by resistant aphids. This, however, does not preclude hitherto undiscovered mutations at this structural locus.

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Spider feeding behaviour optimises dietary essential amino acid composition

GREENSTONE¹ showed that dietary mixing occurs in the lycosid *Pardosa ramulosa* (McCook) and claimed that the spiders optimise their intake of essential amino acids. He took the optimal proportions of amino acids to be those present in the spiders and estimated the ingested nutrients from the amino acid composition of ‘appropriate extracts’ of the prey. I suggest that the extracts he used were inappropriate so that the data cannot be used to test the hypothesis that they forage optimally for essential amino acids.

The appropriate extract used by Greenstone was prey haemolymph “because spiders do not consume their prey intact and therefore do not ingest skeletal material” (see Table 2 of ref. 1). However, spiders pour digestive fluids onto their prey and ingest the fluid products and the haemolymph but discard the cuticle. In *Tegenaria atrica* Koch these digestive fluids contain a wide range of proteolytic enzymes as well as esterases and carbohydrases². A similar range of digestive enzymes must be expected in all spiders.

Except for the haemolymph, the distribution of amino acids in insects is poorly known but the information available shows no correlation between the amino acid composition of insect protein and that in the free pool amino acids³. In addition the amino acid composition varies between tissues as well as between sexes⁴. Consequently the amino acid composition of ingested material will reflect that of both the haemolymph and of those tissues digested externally. The proportion of each tissue digested will vary because spiders consume different amounts of individual prey items according to, *inter alia*, disturbance, prey type, and hunger and/or prey density. The relationship between the amino acid content of prey haemolymph and the food ingested needs

to be established before the hypothesis can be tested.

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GREENSTONE REPLIES—As Humphreys intimates, the most appropriate extract for the dietary studies is not an easily extractable fraction, but rather the difference between the total amino acid composition of the insect and that which remains in the carcass after the spider has fed upon it. At the time the amino acid studies were begun it was not possible to initiate a feeding study. Large stocks of the insects, however, were on hand, from which two types of extracts were potentially available: haemolymph alone, which might underestimate the spiders’ amino acid consumptions, and macerates of whole insects, which might overestimate them. I chose to risk underestimation because the spiders’ evolutionary history has predisposed them to having low prey utilisation efficiencies.

Pardosa species are small and live in open habitats¹; this exposes them to heavy density-independent mortality. *P. ramulosa* spiderlings generally have a high ballooning frequency (my unpublished data), which is a correlate of habitat instability^{2,3}. Open and unstable habitats are r-selecting, and among the expected traits of r-strategists is low food utilisation efficiency⁴. *Pardosa* species have been shown to have lower prey utilisation efficiencies⁵, and jumping spiders to feed for less time on each prey item⁶, when food is abundant, as was the case in the study area during my field work⁷. These facts suggested that the spiders would be more apt to take only haemolymph than to consume all of the extractable amino acids.

The use of haemolymph has the additional advantage that material from a large random sample of an insect population can be pooled, thereby averaging out some of the age and sex differences in amino acid composition.

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BOOK REVIEWS

Food for thought

G. Curzon

BEFORE the 1970s most neurochemists (stimulated by current ideas on biochemical regulation) were probably more interested in brain enzymes than in the substrates they acted on. Consequently, the availability of these substrates to the brain was given relatively little attention. Also, it was thought that, except in severe malnutrition, the brain controlled its nutritional intake so that this was appropriate to its needs. But in the past decade emphasis has shifted, especially with regard to neurotransmitter synthesis. The change began with studies on how brain synthesis of the transmitter 5-hydroxytryptamine (5HT) is controlled. Tryptophan hydroxylase, one of the two enzymes required for 5HT synthesis, is rate-limiting but normally well below saturation with its substrate, tryptophan. This is an essential amino acid, mammals are completely dependent on dietary sources and it is found that normal dietary changes of tryptophan availability to the brain can affect how much of the transmitter is made there.

These findings have led to an explosion of research activity in which Wurtman and his group have played a major role. This interest is strongly reflected in the series so that many chapters describe how dietary constituents get to the brain and their effects on transmitter synthesis therein. Indeed, the whole of Vol. 5 derives from a symposium on the effect of choline supply on brain acetylcholine synthesis. Apart from this, five chapters, distributed between Vols 1, 3 and 4, deal wholly or in major part with dietary or other extracerebral influences on transmitter synthesis. Thus, in Vol. 1, Pardridge describes how amino acid availability to the brain is regulated and Ordóñez similarly discusses folic acid derivatives and choline. Volume 3 contains a chapter by Growdon on dietary neurotransmitter precursors and their use in the treatment of brain disease. Another chapter by Sourkes is on other nutritional factors that may affect transmitter synthesis. Volume 4 includes a chapter by Guroff on how inborn errors of extracerebral metabolism of amino acids and other substances alter the nutrition of the brain.

Nutrition and the Brain. Edited by R.J. Wurtman and J.J. Wurtman. Five volumes. (Raven: New York.) Vol. 1. *Determinants of the Availability of Nutrients to the Brain*, pp.336, 1977, \$29. Vol. 2. *Control of Feeding Behavior and Biology of the Brain in Protein-calorie Malnutrition*, pp.323, 1977, \$29. Vol.3. *Disorders of Eating. Nutrients in Treatment of Brain Disease*, pp.314, 1979, \$29. Vol. 4. *Toxic Effects of Food Constituents on the Brain*, pp.232, 1979, \$23. Vol. 5. *Choline and Lethicin in Brain Disorders* (edited by A. Barbeau, J.H. Growdon and R.J. Wurtman), pp.474, 1979, \$42.

Another major area is covered in Vol. 2 and the rest of Vol. 3. These chapters are on normal and pathological aspects of feeding: control of eating (Vol. 2, Lytle); metabolism in obesity and anorexia nervosa (Vol. 3, Cahill *et al.*); medical aspects of these disorders (Vol. 3, Bruch); a critical analysis of claims for megavitamin therapy in mental disease (Vol. 3, Lipton *et al.*). Most of this material is on effects of disturbances of volition towards eating. The brain obviously has a causal role in these disturbances although little is at present known about its nature. The rest of Vol. 2 is on protein-calorie malnutrition — a primarily involuntary disorder. Here the important questions concern effects of the reduced food intake on the human brain, their reversibility and their behavioural consequences. As Shoemaker and Bloom point out in their chapter on the effect of undernutrition on brain morphology, early undernutrition might conceivably affect subsequent behaviour and other brain functions by numerous mechanisms. While present evidence for specific mechanisms may well be unclear this does not diminish the enormous potential social importance of research in this area. Other chapters in Vol. 2 are on biochemical (Nowak and Munro) and behavioural (Pollitt and Thomson) effects of protein-calorie malnutrition. Background material on the dietary patterns of numerous human societies and of non-human primates is given in Vol. 1 by Gaulin and Konner.

Tables 7–9 are particularly fascinating.

Volume 4 is largely on toxic effects of food constituents, i.e. food additives and hyperkinesia (Lipton *et al.*), glutamic acid (Garattini), peptides (Zioudrou and Klee), alcohol (Tabakoff *et al.*). These effects range from the obviously important but imperfectly understood (alcohol) to the probably unimportant (glutamic acid), although it is necessary to keep in mind the conclusion of the chapter on hyperkinesia that there is probably no food or drug without some toxicity to some individuals.

Volume 5 is substantially different from the other volumes, which each contain four to six chapters, as it contains 38 contributions to a symposium. Topics include choline, lecithin and acetylcholine metabolism, and the pharmacology of the cholinergic neurone. The main focus is on the extent to which functional brain cholinergic activity is affected by dietary supplements of choline and lecithin and whether these compounds can be used to treat various disorders of the central nervous system. There seems to be some evidence that large dietary supplements may alleviate tardive dyskinesia but not other dyskinetic states such as Huntington's chorea and dopa dyskinesia. Patients with presenile dementia, though they have a major defect in brain acetylcholine synthesis, show disappointingly little response. In general, the therapeutic trials described were of a preliminary nature. Neither of the studies on tardive dyskinesia was done 'blind'. The chapters by Ansell and Spanner, Haubrich *et al.* and Karczmar are particularly useful, as they provide a biochemical and pharmacological background for assessing the (on the whole less substantial) clinical papers which take up much of the volume.

The series in general is strongly recommended. It is attractively produced and well indexed. The overall clarity of presentation must indicate much hard editorial work. One may expect these volumes to be a major influence on future research in their field. □

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Methods of polarographic analysis

Emil Paleček

Polarography of Molecules of Biological Significance. Edited by W. Franklin Smyth. Pp.328. (Academic: London and New York, 1979.) £22.60, \$52.

THE sensitivity of polarographic (voltammetric) determinations has greatly increased in the past 10-15 years. While the limits of detection of organic and inorganic substances reached by classical d.c. polarographic methods were usually around 10^{-5} M, using modern differential pulse anodic stripping voltammetry, inorganic substances can be determined at concentrations down to 10^{-10} M. Cathodic stripping voltammetry is becoming a very useful tool in the analysis of certain organic compounds at concentrations as low as 10^{-8} – 10^{-9} M; and differential (derivative) pulse polarography operates at concentrations of 10^{-6} – 10^{-8} M for both organic and inorganic substances. This remarkable gain in sensitivity, together with the commercial availability of reliable polarographic analysers, has resulted in a steady increase in the number of analysts using modern polarographic (voltammetric) methods. This process, sometimes called the 'renaissance of polarography', is, however, not yet accompanied by the predictable abundance of books concerning the theory and application of modern polarographic methods. Smyth's book is thus a valuable acquisition for analytical chemistry libraries.

The first two chapters of this book describe unit processes in polarographic (voltammetric) analysis of organic substances such as choice of method, preparation of the sample, selection of a proper supporting electrolyte, cell and electrodes, derivatization and complexation procedures, and so on. Further chapters deal with the application of polarographic methods in pharmacy and pharmacology, basic medical and environmental sciences, and agriculture. Polarographic determinations of various groups of substances are in general compared with determinations by means of other methods; and the advantages of the polarographic analysis of certain types of compounds are stressed. It is shown that differential pulse polarography is highly suitable for the determination of pharmaceutical products and other substances containing nitrogroups; and sulphur-containing substances (many of them representing trace foreign materials which pollute the environment) can be determined at low concentrations by cathodic stripping voltammetry.

The book is well-written; it has not,

however, managed to escape entirely without errors; for example, in the paragraph on purine and its derivatives (Chapter 5) it is stated that pyrimidine and cytosine are oxidizable at graphite and mercury electrodes. It follows from the cited paper (G. Dryhurst and P. J. Elving) that both substances are non-oxidizable. In spite of the fact that a relatively extensive literature on polarography of nucleic acids exists, only two original papers are cited in Chapter 6, one of them incorrectly; review articles concerning this topic are

completely ignored.

Minor errors do not, however, substantially diminish the significance of this book, which can be confidently recommended to all analytical chemists dealing with determinations of biologically important organic substances, and especially to those of them who have come in contact with polarography in the past. □

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Interstellar dust

D.E. Brownlee

Cosmic Dust: Its Impact on Astronomy. By Peter G. Martin. Pp.266. (Clarendon/Oxford University Press: Oxford, 1979.) £9.

PETER MARTIN'S *Cosmic Dust: Its Impact on Astronomy* is not an encyclopaedia of what is known about extraterrestrial dust. It does not cover Moon dust, Mars dust, comet dust, Venusian smog or plumes from Io's volcanoes. It is a book which concentrates on star dust and is a comprehensive coverage of current information and ideas concerning dust in the interstellar medium. Interstellar dust is a basic form of matter in galaxies and is the common form of most elements heavier than helium that are not currently residing in stars. Interstellar grains are produced in gaseous regions surrounding stars; they experience an evolutionary existence as tiny astronomical bodies and are eventually destroyed by super novae shocks or interactions with stars. They are somewhat of a nuisance, as they totally obscure most of our Galaxy in visual wavelengths and cause extinction, reddening and polarization in observable objects. However, as Dr Martin enthusiastically points out, dust grains are fascinating bodies important to a variety of disciplines in astronomy.

The book's first five chapters deal with the interaction of dust and radiation in the interstellar medium. These chapters cover fundamental theory as well as up-to-date observational and laboratory data. Topics include radiation transfer, calculation and laboratory measurement of particle cross-sections, extinction of optical and X-ray wavelengths, polarization, and scattering. The fifth chapter deals with the properties and modelling of reflection nebulae. In addition to providing the reader with a good background on the interaction of light and dust, the author also interprets existing observations in terms of limitations they place on the nature and distribution of interstellar dust.

Chapters six and seven discuss the theory and observation of thermal emission from grains. This includes far-infrared from the

Galactic plane and the properties and structure of circumstellar dust shells. Chapters eight and nine deal with the distribution of dust in the Galaxy, the nature of dust clouds and interactions of dust with the interstellar environment. Specific topics include radiation pressure, spin, charge, sputtering, molecule formation, alignment and dust in dark clouds, and H II regions, planetary nebulae and the Solar System. The coverage of most of these topics is somewhat introductory and does not go into considerable depth. In chapter ten the significance of spectral signatures such as the 2200 Å and 10 µm features are discussed as indicators of grain composition. Also discussed are the importance of cosmic abundances and abundances in interstellar gas as constraints on grain composition. Chapter eleven reviews the evolutionary processes and timescales experienced by dust. Basically this chapter is concerned with the formation of refractory cores and volatile mantles and their stability in changing interstellar environments. The final chapter contains a brief section on dust in other galaxies and an intriguing discussion about properties and possible observations of dust between galaxies.

In the preface the author states a goal to give the reader basic tools and to discuss concepts underlying current research. This he does quite well. A careful reading of this book will provide the reader with a broad-based background in both the theory and current state of observation of interstellar grains. In general the book can be highly recommended, although there are some minor drawbacks. The organization is such that certain topics are diffused through widely separated parts of the book. It sometimes is difficult to answer a specific question without reading most of the book. Although it provides a good background, this is not a handy source book for quick reference. The most serious drawback in the opinion of this reviewer is the lack of formal references. At the end of each chapter is a list of "further reading" but these are not true references that refer to specific points in the text. This continually raises a question of credibility. The reader is apparently expected to accept the written word as dogma. The lack of conventional references also makes it difficult for the

reader to go into a specific subject in more depth than covered in the text. Annoying but less serious problems are with the figures. Most of the line drawings are excellent and were printed in a readable standard format. A small number of drawings, however, are direct computer plots with tiny difficult-to-read labels and needless excess zeros behind the decimal point. Many of these are multifunction plots where individual lines are very difficult to distinguish from each other. Only a few plots are really terrible but they are certainly not for the farsighted. Some

of the letters are less than $800\mu\text{m} \times 500\mu\text{m}$, some of the plotted lines are actually less than a $100\mu\text{m}$ in width, and in places both plotted lines and figure borders totally disappear. The continuous tone photographs have poor tonal quality similar to newsprint.

Cosmic Dust is a comprehensive up-to-date book that will be of significant value to anyone working in fields involving interstellar grains. It contains a good coverage of the theory, observations and current state of understanding of dust in the interstellar medium. For someone just

getting into this interdisciplinary field it contains a wealth of suggestions for important future work. For the corner librarian this book will be a headache. Although *Cosmic Dust* is the first *Cosmic Dust* devoted entirely to interstellar dust, it is the third book with a jacket label of this title published in the past 15 years. □

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Forest ecosystem

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Pattern and Process in a Forested Ecosystem: Disturbance, Development and the Steady State Based on the Hubbard Brook Ecosystem Study. By F.H. Bormann and G.E. Likens. Pp.253. (Springer: Berlin, Heidelberg and New York, 1979.) DM42, \$23.10.

As the sub-title indicates, this book describes the work of the Hubbard Brook Ecosystem Study in northern New England. The authors' objective is to provide an integrated picture of the structure, function and development of an area of northern hardwood forest and to set their data against current concepts of ecosystem dynamics. In effect, this book is one of the most complete accounts of secondary woodland succession following clear felling so far to be produced.

The authors propose a four-phase biomass accumulation model of woodland development of reorganization, aggradation, transition and steady state. This differs from the familiar smooth-curve biomass accumulation model of Odum in that the initial 'reorganization' phase of up to 15 years is a period of net loss in biomass when total ecosystem respiration exceeds GPP, as is also the transition phase which precedes the final 'steady state', when total biomass oscillates about a mean. Most of the book is inevitably concerned with a discussion of the quantitative characteristics of the 'reorganization' and 'aggradation' phases as these have been the subject of the long-term study. Here can be found a wealth of data on biomass accumulation, productivity, hydrological and mineral cycles, species diversity and evolutionary strategies. The discussion of the 'transition' and 'steady-state' phases is necessarily speculative as no examples of primaevial forest survive in New England and the authors estimate that in the absence of external catastrophic events it takes about 300 years for the steady-state phase to be reached by natural developmental processes.

Many authors in the last twenty-five years have questioned the classical Clementsian view of 'climax' vegetation, insisting that it represents no more than an unrealizable abstraction. This is based on the assertion that natural succession is inevitably truncated somewhere in the aggradation phase (of the present authors) by cyclical catastrophic events such as fire or wind-blow. This issue is fully examined in the light of the available historical and ecological evidence, and the authors conclude that so far as their study area is concerned the fire-rotation periods are far longer than would be needed to prevent natural development reaching the steady-

state phase and that climax forest would indeed have been a reality in pre-settlement times.

Throughout the book there are undertones of a Clementsian 'supra-organism' philosophy but these are not permitted to become unduly obtrusive. The authors freely acknowledge that much more is owed to A.S. Watt's concepts of pattern and process in vegetation, an approach that will meet with a sympathetic hearing from most British ecologists. □

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Sediment studies

R.C. Selley

Coastal Sedimentation. Edited by D.J.P. Swift and H.D. Palmer. Pp.339. (Dowden, Hutchinson and Ross: New York. Distributed by Academic: New York and London, 1979.) \$34, £22.50.

In the words of Professor Fairbridge, Editor of the *Benchmark Papers in Geology*, the philosophy behind the series is "one of collection, sifting and rediffusion". The basic concept is to republish selected seminal papers on a chosen topic in their original form (with minor excision) enveloped in editorial comment.

This is designed to overcome the problems of a scientist trying to delve into original literature only to find that the important references are diffused through many journals, or that, in the case of the newer centres of learning, runs of journals are of too short a span to contain vintage papers.

The first of these problems is particularly acute in the field of coastal sedimentation which is reviewed in this particular benchmark volume. This is a topic which is of interest to a wide range of scientists, engineers and even ecologists and conservationists. These range from geographers, geologists, civil and

hydraulic engineers to marine ecologists and sailors. Thus, it is not surprising to find that the papers in this volume are reprinted from periodicals as diverse as the *Journal of Waterways and Harbors Division of the American Society of Engineers*, the *Bulletin of the Georgia (USA or USSR) Academy of Sciences*, the *Bulletin of the American Association of Petroleum Geologists*, the *Beach Erosion Board of the US Army Corps of Engineers*, and a symposium volume published by the National University of Mexico. Few scientists can claim to have such eclectic interests as to monitor all these publications. They must be too busy reading to do any work if they can.

The book reprints a total of twenty papers which have been arranged in four groups. Each group is preceded by several pages of comment and introduction by the editors.

The papers in Part I are concerned with the coastal equilibrium profile. They begin with a bowdlerized version of Fenneman's classic paper of 1902, twenty pages having been omitted from the original thirty-two. This is followed by several papers written by US geologists in the nineteen-sixties. Part II is concerned with coastal deposits, reprinting five papers describing certain North American beaches and shelves. Part III is headed "Studies of Fluid Motion" and reprints five papers ranging from "Bottom Currents during Hurricane Camille" to "Wind-Driven and

Thermohaline Circulation over the Continental Shelves'. Part IV contains four papers on the theme "Studies of Substrate Response". These present observations on the erosion, transportation and deposition of sand in selected coastal areas. The book concludes with author and topic indexes.

From the preceding account it is clear that the titles of this volume should perhaps not have been so catholic as *Coastal Sedimentation* but rather 'North American Sandy Coasts'. No two scientists would produce the same top-twenty list of significant papers on coastal sedimentation. Nevertheless, the concentration on sandy coasts

of the USA is surprising. Only two papers deal with non-US coasts, one in Mexico, the other in the North Sea. There are no papers on carbonate coasts, either of such classic areas as the Bahamas or the Persian Gulf. Neither are there any papers on deltas, or on muddy coasts, such as the tidal flats of the North Sea made famous by Dutch and German workers.

A review of previous benchmark volumes shows that coastal sedimentation has already been done in various disguises — No.3, Spits and Bars, No.9, Barrier Islands, No.30, Holocene Tidal Sedimentation, and No.39, Beach Processes and Coastal Hydrodynamics. Perhaps some of

the omissions noted in this book have appeared previously. A volume entitled 'Benchmark Papers of Benchmark Papers on Coastal Sedimentation' is perhaps now overdue.

It is hard to see this particular book appealing to many individual scientists. Perhaps only the newest and wealthiest of libraries may consider purchasing it. Since the text has been prepared by photographically copying the original papers, some in typescript, the cost of £19.15 seems rather high, perhaps even a benchmark. □

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Glucocorticoids at work

G. S. Boyd

Glucocorticoid Hormone Action. Edited by J. D. Baxter and G. G. Rousseau. Pp.638. (Springer: Berlin, Heidelberg and New York, 1979.) DM 118, \$64.90.

THERE is interest in the mode of action of the steroid hormones in attempts to discover at the molecular level how these hormones work. Investigation of the way in which sex hormones act is simpler since it is possible to identify specific target tissues. However, the glucocorticoids present a difficult challenge because these steroids have such widespread effects, and the tissue response to the hormone depends upon the nature of the tissue.

The late Gordon Tomkins played a significant part in attempts to discover how the glucocorticoids work, and it is fitting that this volume should be dedicated to the memory of this brilliant researcher.

The book is a series of short essays on various phases of the possible mode of action of glucocorticoids. The editors have contributed an introductory chapter, various other chapters and a concluding generalizing chapter updating some of Tomkins' concepts and placing these views in a modern setting. The volume has over 600 pages and is divided into 34 chapters so that any one contribution is fairly short. The contributors appear to have been adequately briefed by the editors because there is a reasonable degree of continuity throughout this multi-author review. The book contains accounts of the transport and uptake of corticoids by cells. Various aspects of the association of the hormones with the cytosolic receptor molecules are expounded, the structure-activity relationships are explored, and the translocation into the nucleus and the nature of the nuclear binding of the glucocorticoids are considered. The grey areas on the activation of the genetic

apparatus in the nucleus are critically reviewed and the role of the glucocorticoids as important factors involved in the induction of tyrosine aminotransferase and tryptophan oxygenase is considered. It seems a pity that the chapter on tryptophan oxygenase is one of the shortest in the book. In separate chapters various authors deal with the mode of action of corticoids on fibroblasts, thymocytes, leukaemic cells, pituitary cells and leukocytes. There are also contributions on the role of glucocorticoids in glycogen metabolism,

lipid metabolism, insulin action and the stability of lysosomes. The editors have made a bold attempt to obtain coverage of as many aspects of corticoid action as possible in this sizeable book. Naturally this has resulted in a degree of overlap in places. Nevertheless this is a good book which will be a useful source to all who wish to have ready access to information on the action of the glucocorticoids up to about 1977-1978. □

G. S. Boyd is Professor of Biochemistry at Edinburgh University Medical School, UK.

Collision theory

A. D. Buckingham

Atom-Molecule Collision Theory. Edited by R. B. Bernstein. Pp.779. (Plenum: London and New York, 1979.) \$57.50.

IN the last 20 years there has been an enormous growth of interest in the study of molecular collisions by beams techniques. In 1977 the American Chemical Society was able to arrange a symposium entitled "State-to-State Chemistry". The advances of electronic and vacuum instrumentation have produced a wealth of information on atomic and molecular cross-sections covering elastic, inelastic and reactive scattering. This volume is intended as a guide to scattering theory for experimentalists in the field of molecular collisions. It is restricted to atom-molecule collisions, for which the theory is at present tractable.

The first chapter is by Bernstein himself. It provides an interesting and informative overview of the development of the beams technique and of the current state of experiment and theory. The second chapter, by H. F. Schaefer III, reviews the *ab initio* computation of potential energy surfaces; the third, by P. J. Kuntz, is on semiempirical potential surfaces for collision theory. The analysis of elastic

scattering is discussed in great detail by H. Pauly for central forces, and by S. Stolte and J. Reuss for non-central forces. Inelastic scattering theory is covered in chapters by J. C. Light, D. J. Kouri, D. Secrest, M. D. Pattengill and W. R. Gentry. Non-adiabatic electronic transitions are described by M. S. Child, and reactive scattering by J. C. Light, R. E. Wyatt, D. G. Truhlar, J. T. Muckerman and D. A. Dixon. There are chapters on collision-induced dissociation by D. J. Diestler and P. J. Kuntz, and the final contribution is by R. D. Levine and J. L. Kinsey on the application of information theory to molecular collisions. There are 22 chapters and author and subject indexes, making up the 779 pages.

The book has some of the usual problems associated with a multi-author volume; however these are not grave, and the overlap and the changes in notation are not really a serious disadvantage. As compensation the spread of authorship, including many distinguished contributors, has provided a breadth of coverage that no single writer could have achieved. The book is highly recommended to those for whom it was intended — experimentalists in the field of molecular scattering. It will also be valuable to a wider group of physicists and chemists interested in the dynamics of molecular collisions. □

A. D. Buckingham is Professor of Chemistry at the University of Cambridge, UK.

17 April 1980

Conserve energy, conserve cash

"YOU can't run Britain like a grocer's shop!" said an exasperated trade union leader recently. He was referring not only to Mrs Thatcher's humble economic education, but also to the government's uncompromising arithmetic policy of restricting current expenditure by taking pennies, regardless, off every item.

The only lines in Britain's shop to remain untouched have been ones close to Mrs Thatcher's heart: defence, law and order, and nuclear power — the latter benefiting from a £10 billion spending programme over the next decade. Energy conservation, on the other hand, she has been content to leave to market forces.

Officials in the Department of Energy are not impressed, according to an internal memo recently leaked to the media, which draws attention to "a sharp and visible contraction in the government's conservation programme". The problem, says the memo, may be that "apart from improving the building regulations, we have in fact no proposals on the conservation front which do not call for extra resources either in terms of money or staff. At the present time, both are clearly not starters."

Another leaked document — this time minutes of a meeting of the 'Committee of Ministers on Energy Conservation' — shows that the junior minister concerned with conservation, Mr John Moore, is unhappy with Prime Ministerial attitudes. "Mr Moore" say the minutes "felt the committee could achieve little without the evidence of a political will from [Mrs Thatcher]".

The UK has not, in the past, come out well in international comparisons of measures taken to conserve energy, and it seems that the country is slipping further down the league. In its orgy of cutting spending, the government has:

- scrapped plans for 14 regional home energy saving advice centres (saving £500,000 a year)
- announced the closure in June of a scheme to help businesses improve inefficient boilers, insulate buildings, and install or improve combined heat and power schemes (saving £25 million over 2 years)
- halved the budget for assisting individuals to insulate their homes (saving £12.5 million).

But conservation needs more than the price mechanism alone. As the Secretary of State for Energy, Mr David Howell, said last year, there has to be "sensible and sensitive" government intervention or the energy conservation programme will be unbalanced. Pressure is

rising on Mrs Thatcher to take cognizance of this. Last month a syndicate of ten organisations including the Electricity Consumer's Council, the increasingly important Parliamentary Liaison Group for Alternative Energy Strategies, and other bodies with a long record of serious concern with the energy scene, requested that the Select Committee on Energy should turn its attention to the effect of spending cuts on the conservation programme; and it seems likely that it will follow their lead. The committee should do so, as a matter of priority, for the leverage on primary energy consumption of a pound spent on conservation is probably very great.

This is to say that a pound spent on conservation can save more energy than a pound spent on production can produce. According to some estimates, the factor can be as high as three. And judging by French plans (reported in *Le Monde*) to save 10 million tonnes of coal equivalent by spending £6 billion in industry, the French government itself — despite its ambitious nuclear programme — is aware of these relativities.

David Howell, opposition spokesman on energy, told *Nature* this week "Britain spends peanuts on conservation. We want a step-change in attitude". And the attitudes we need to change are those which see energy production as a matter of investment, but energy conservation as a matter of giving grants. The Central Electricity Generating Board, the Gas Board, the National Coal Board actually advertise to encourage us to use more energy: 'cook electric' advertisements appear regularly on television despite the fact that electric heating consumes three times the *primary* energy of the equivalent gas heater or cooker. Power stations are only one-third energy efficient.

As the Department of Energy memo has it: "If the funds spent on conservation replace the same or greater amounts spent on supply, should it matter that they are going to private companies or individuals rather than, say, the CEEB or the NCB?" Certainly not if the true blue objective of an energy policy is to ensure that the economy is unrestrained by constraints on energy supply. The economy can be released from energy costs more rapidly, more continuously (with less lumping of capital, unlike the £1 billion per nuclear reactor), and most likely, more cost-effectively. If you conserve energy, Mrs Thatcher, you conserve cash; and you conserve it rapidly, with payback times that are within the lifetime of your parliament. Is that not sufficient political incentive? □

The Antarctic

Managing krill in the Southern Ocean

THROUGHOUT next month, the thirteen signatories of the Antarctic Treaty will meet in private in Canberra, Australia to negotiate a convention to control the exploitation of krill — commonly regarded as the Antarctic's most immediately exploitable resource. Krill are small shrimp-like creatures which thrive in great abundance in the extraordinarily nutrient-rich cold waters of the Southern Ocean. They are central to maintaining the ecological balance of the Antarctic because they feed on abundant phytoplankton and in turn are fed on by many larger animals; in particular they form the staple food of whales. They are also increasingly being recognised as a potential source of protein for man. Hence the need for a convention which controls man's exploitation in such a way as to be non-discriminatory between different nations and at the same time maintain the local eco-system.

The Antarctic Treaty states have been negotiating the 'krill convention' — Convention on the Conservation of Antarctic Marine Living Resources in secret — for several years. Next month's meeting is hoped to be the last: agreement on the text of the convention should be reached, although ratification could take up to ten subsequent years.

The success of the convention, however, will also depend upon how acceptable it is to other nations not involved in its negotiation but nevertheless interested in reaping some of the benefit of the opening up of new fishing grounds. In particular, several developing countries have expressed fears that the convention only takes into account the interests of the

Antarctic Treaty signatories and other countries which are technologically capable of fishing in the extreme conditions of the Antarctic. The secrecy of the negotiations has not helped to dispel such fears and some observers, in particular the authors of a report published by the International Institute for Environment and Development (IIED)* last week, believe that agreement on the convention may spark off a wider international debate on the rights of access to the fishing grounds.

Reaching some sort of agreement just among the Antarctic Treaty signatories, however, will not have been easy. The key problem has been how to cope with the territorial claims of some nations to large slices of the Antarctic continent together with 200 mile-wide coastal strips of sea. Those signatories who have not laid claim to land do not recognise the claims of the others. The problem has therefore been to work out a 'bifocal' approach which leaves open the question of sovereignty over coastal fishing grounds.

The political problems of negotiating the convention are certainly great — but there are also many scientific problems to be solved before a system of good management for the Southern Ocean can be established. As yet there are no reliable estimates of the amount of krill which could be caught annually on a sustainable basis. There is also insufficient information on the interaction of different species living in the area.

The scientific problems implicit in the krill convention are different from those associated with other fisheries agreements

because of the nature of the convention's prime objective — the conservation of marine living resources. This is a totally new approach to fisheries control according to Dr John Beddington of York University who helped the IIED prepare its report. Previous agreements have mainly been concerned with the optimization of harvesting by man.

Although Dr Beddington regards the spirit of the convention on conservation as worthy, he is critical of its scientific approach. In particular, it does not take into account the fact that the net greatest annual increment in a population can be achieved only by removing its predators. In the case of krill this would mean killing whales — a contradiction of the convention's aim of allowing depleted whale populations to recover.

The IIED report recommends alterations to the wording of the convention to eliminate this contradiction. It also suggests that the scientific committee which is to be set up under the convention to provide scientific information, should maintain close links with independent scientists. However, the BIOMASS (the Biological Investigation of Marine Antarctic Systems and Stocks) programme, managed under the aegis of the International Council of Scientific Unions, should remain independent from the scientific machinery of the convention, it says. It should supply independent scientific information, free from any political constraints.

Judy Redfearn

**The Management of the Southern Ocean
Barbara Mitchell and Richard Sandbrook,
IIED, 10, Percy Street, London W1P 0DR. £2.*

Energy

France plans one third nuclear energy by 1990

A confident forecast by the French Ministry of Industry has predicted that by 1990 France will have cut its dependence on oil to one third of its primary energy demand. It will do this, says the ministry, mostly by a dramatic ten-fold increase in the use of nuclear power, but also through contributions from gas and renewable energies — particularly solar and geothermal. The renewable contribution must increase four-fold in the next ten years to meet the ministries' targets.

France already makes considerable use of hot springs for household heating. All renewable resources (other than hydro) together now contribute only 1.5% of France's annual 323 million tonnes of coal equivalent energy demand, but, says an official communiqué of the council of ministers "certain renewable resources will come to maturity" in the next decade.

These projections considerably exceed those, say, of the UK, where energy

minister David Howell told the Select Committee on Energy recently that "the contribution from renewable sources in the year 2000 might be up to 10 mtce. My department now considers that this is, if anything, an overestimate". 10 mtce would be 2.5% of total energy use in 2000.

UK nuclear projections are also much smaller, a doubling to 9% of demand by 1990, compared to France's 30% by the same date. France must build one reactor every two months to reach its target.

According to *Le Monde* (4 April) the government expects that a £6 billion investment in energy conservation in industry by 1990 will save 10 mtce per year.

Moreover to encourage those living near the sites of projected nuclear power stations to accept their lot, electricity supplies will be set 12 to 17% cheaper for those living "in the vicinity" of the power station.

However not all are happy with the

plans. *Le Monde* quotes M. Michel Rolant, national secretary of the giant trade union CFDT, as saying that while it is desirable to reduce dependence on oil "it is dangerous to make this objective dependent on the imposition of electrical energy on all domains other than transport". The plans imply that in 1990 42% of French primary energy will be converted to electricity, 73% of that being produced by nuclear power stations. At present about 30% of French primary demand goes to electricity generation.

Moreover, said Rolant, France is taking a formidable bet on the good performance of the 66 or so nuclear reactors it will need in operation by 1990.

The cost of this programme — £26 billion according to Rolant — "is so large that it will make it impossible to follow a serious programme of energy conservation, or of the development of renewable sources . . ."

Robert Walgate

BIOGEN, the European-based genetic engineering firm, has had 600 "very good quality" applications for 18 post-doctoral positions in its new Geneva laboratory, Biogen's President, Robert Crawthorne, said last week. The applications come from throughout the world, but it seems likely that one third of the posts will be filled by applicants from continental Europe, one third from the UK, and one third from the US.

"The numbers we are looking for won't drain the pool of available talent" said Crawthorne "but we're looking for the best". Other companies may have had greater difficulty in recruiting, but Biogen has had something special to offer, he said: a chance to work with a scientific board of ten top academics in molecular biology (seven in Europe and three in the US) and "strong and open" links with the universities.

Biotechnology

600 apply to Biogen

Biogen is also planning greater links with European companies, particularly in chemical, petrochemical, and process engineering, said Crawthorne. The first cooperative agreement is expected shortly. "We already have a strong European shareholding: 36%, with 41% in the US and 23% in Canada. But we are not looking for shareholders any more." Rather, Biogen wants to learn from relevant industries what might be the most profitable products to develop with genetic engineering. "We've got expertise in recombinant DNA" said Crawthorne. "The problem is knowing what line to work on."

Biogen has found it very profitable to work with the US pharmaceutical company, Schering Plough, to whom it has licensed interferon production based on the successful cloning and expression of the leucocyte interferon gene by one of Biogen's scientific board, Professor Charles Weissman of the University of Zurich. "We want to extend such links" said Crawthorne, in Europe and elsewhere.

On interferon, he said Biogen was nine months from producing enough for clinical trials. A number of laboratories may undertake pilot plant production, including Professor Brian Hartley's laboratory at Imperial College, London, but decisions on such matters would be undertaken in consultation with Schering Plough. However, there were still a lot of hurdles, particularly over yields and purification.

Robert Walgate

Soviet Union

Moscow seminar meets

EARLIER this week, several non-Soviet scientists gathered in Moscow with about 50 of their Soviet colleagues for the fourth annual "International Conference on Collective Phenomena". These conferences, which are held outside the usual rigid framework of scientific exchanges, grew out of the weekly "Sunday Seminars for Refuseniks", which began in 1973 to provide some kind of intellectual life for Jewish scientists who, having applied for a visa for Israel, found themselves denied professional employment.

Under their restricted conditions of work, without access to libraries, laboratories, computers and the like, it is, inevitably, the mathematicians and theoretical physicists who have the best chance of producing meaningful research. The majority of scheduled papers, therefore, were of a mathematical nature. Indeed, the hosts of the conference, Viktor Brailovskii and his wife Irina, are themselves both mathematicians.

One of the participants, Aleksandr Ioffe, since losing his academic post in 1977 has published several papers in foreign journals, including the *Transactions of the American Mathematical Society* and the *Comptes Rendus* of the Académie Française. In February, 1980, his foreign colleagues held a two-day mathematical symposium in his honour at Imperial College, London, where all but one of the papers were developments of Ioffe's work.

Ioffe, unfortunately, is something of an exception among the refuseniks, most of whom can have little contact with the scientific community abroad. The "Collective Phenomena" conferences (as opposed to the more domestic seminars) were founded

precisely to overcome this feeling of isolation. This year's seminar was sponsored by the UK Institute of Physics, the New York Academy of Sciences, the French Physical Society, the French Institute of Chemistry and the Norwegian Physical Society, inspired, one may assume, by feelings of scientific solidarity rather than the hope that any major new research would be presented there.

The same solidarity, since the conferences began, has encouraged several western scientists each time to make the journey to Moscow, and to present papers of their own. Under Soviet conditions,

organizing an "independent" scientific meeting is liable to run foul of the authorities. Although some intending participants were refused visas, those who actually reached Moscow did not meet with the particular brand of bureaucracy directed at Dr Anthony Kenny, Master of Balliol College, Oxford, last weekend. His lecture, at the underground "Patocka University" in Prague (which met in the flat of Dr Julius Tomin, the dissident philosopher) was interrupted by the security authorities, on the grounds that his visa had been given for tourism, not for addressing other scholars. □

Spaceflight record-holder in orbit again

VALERII Viktorovich Ryumin holds the current record for orbital flight (175 days). His return last week to Salyut-6, where he established this record last year, has caused certain speculation abroad that the Soviets might be preparing a manned mission to Mars.

Such a mission, in fact, would conflict with all "classical" Soviet projects for space exploration. These, following Tsiolkovskii, the "father of Russian

cosmonautics" envisage the establishment of a permanent orbital station as a necessary preliminary to any deep-space mission. Such a station, it is understood, would be staffed by "several tens" of male and female crew and scientists for shifts of several months at a time. At least for this century, it is understood, lunar and planetary exploration will be left to the much cheaper automatic rover vehicles.

Nevertheless, Soviet space-medicine experts are showing considerable interest in the biological effects of long-term space-flights. Last month, Academician Oleg Gazenko told TASS that the most important result to date was that no significant organic changes had been revealed which might limit the duration of future flights, and that people could work in space for six-month periods.

No doubt Ryumin, with last year's record-breaking flight to his credit, as well as a brief mission in October 1977 aboard Sayuz-25, will prove an especially interesting "subject" — albeit a somewhat serendipitous one — during his current flight on Salyut-6. For, so far from being part of a Mars-preparation programme, he was, according to TASS, only included at a very late stage, when the original candidate, Flight Engineer Lebedev, injured his leg during final training.

Vera Rich



United Kingdom

18% photoefficiency claimed in BP energy research prize

FIXED photochemical energy amounting to 18% of visible light has been claimed by one of the recipients of a new energy research prize last week. The figure compares favourably with the electrical efficiency of solar cells, and is so far beyond the 3% normally regarded as the maximum achievable by plants that some specialists are highly sceptical about it.

The prize — £13,000 — comes from the oil multinational British Petroleum, which announced a £1.5 billion a year capital investment programme in its annual report published last week. "In the future" says the report "an increasing proportion of new investment, research and enterprise will be directed to activities additional to oil and gas". The BP energy prize is considered to be a way, said a BP spokesman, "to begin to get in on the act" on increasingly interesting energy research in the universities. But BP will not claim ownership of any of the results.

BP has awarded £39,000 to be divided between three groups from four UK universities. Similar awards will soon be made in ten other countries: Germany, France, Belgium, Denmark, Holland, Switzerland, Greece, Portugal, Canada and New Zealand. After a year's research, reports from the three groups in each country will be considered, and a national winner selected — who will receive a second year's grant and a cash prize of £5,000. These winners will also be considered by an international panel for an international energy prize of £10,000 to be awarded in July 1982.

The UK winners of the first leg are: Professor S J Pirt of Queen Elizabeth College, London, to develop an algal bioreactor for fixing solar energy; Drs H A O Hill of Oxford and I J Higgins of Kent Universities to improve the efficiencies of fuel cells powered by enzymatic reactions; and Dr R P Howson of Loughborough University to develop optical coatings for improved heat retention by windows. The prizes were awarded by a panel of five drawn from the Royal Society and the Fellowship of Engineering.

Professor Pirt hopes to build a 0.5m² collecting area 'unit' bioreactor, which would be deployed in multiples over a solar collecting field. The collecting surface will be tubular, and carry a continuous culture of *Chlorella* fed by ammonia, salts, and 100% CO₂ ("which" says Professor Pirt "we have discovered is not toxic, contrary to expectations").

The system has been developed in the laboratory. The next stage involves scale-up, and the application of microprocessor control to adjust nutrients and flow rates for varying sunlight intensity.

"Our target is to fix 14% of solar energy incident on the field", Professor Pirt said last week. "We have reached 18% photoefficiency in the laboratory" he claimed. Asked if this was not a very high figure, he said there had been no reliable and consistent data for photosynthetic efficiency. "Figures vary by a factor of four". (Pirt has published his results in *J. Chem. Tech. and Biotech.* 30, 25-34; 1980).

Photosynthetic processes were not at their most efficient at the CO₂ levels present in air, said Pirt. It had proved crucial to provide pure CO₂ to the organism "despite the fact that the books say more than 5% is toxic".

Pirt is "quite confident" that the capital costs of the reactor can be brought below those of competing solar technologies, such as solar cells.

One potential advantage of his closed system — where all external factors other than sunlight and temperature can be closely controlled — is that evolved oxygen can also be collected, and would become a by-product of the process. Moreover collected energy — in algal biomass — would be automatically stored, unlike the electrical energy from solar cells.

The biomass could be fermented to methane to provide natural gas; but fixed nitrogen would have to be recovered if the process were to be overall energy efficient. So "there are a number of other biotechnologies to be appended to this system", said Pirt, "before it is complete".

However, other biomass specialists are highly critical of Pirt's results. One pointed out that the currently accepted, "rock-hard" photosynthetic pathway proposed by Dr Robin Hill of Oxford 20 years ago required 8 photons to fix one CO₂ molecule. This leads to a theoretical maximum energy efficiency of 12%; in practice the record is sugar cane's 3%. But Pirt claims 18%, with a mixed culture of his alga and three heterotrophic bacteria.

"Pirt has claimed that one can produce 150 tonnes per hectare per year, but the best practice anywhere else is 30 to 50," said the biomass specialist. "He is doing biomass a fantastic disservice by making these claims" he said.

Pirt said last week that the accepted photosynthetic pathway "lacked any sound energetic data in support of it". His data "implies that we must look at what is wrong with the pathway" he said. "The gaps in our knowledge of it are really enormous. Otto Warburg [Nobel Laureate for medicine, 1931] always claimed that 4 photons per carbon was the correct figure. Others claim 12."

Robert Walgate

Hungary

Paks power station secured in concrete

HUNGARY'S nuclear power stations at Paks, the first generating set of which will go on stream in 1981, is so safe that not even an earthquake presents any serious danger. So said MP Miklos Vida, recommending a new nuclear energy bill to the Hungarian National Assembly last month. On behalf of the parliamentary committees which had dealt with safety questions, Vida noted that "the nuclear power station in the Armenian SSR which resembles the Paks power station easily withstood the force 5 earthquake . . . of 1976; according to estimates it can withstand force 9 earth movements".

Nor, said Vida, is there any fear of a nuclear explosion. "The system of construction of our nuclear power stations is such that it is a physical impossibility for a chain reaction to get, so to speak, out of hand". The only possible danger would be from "harmful radioactive materials finding their way into the environment". And "all necessary measures" have been taken to guard against this. The spent fuel "which represents the greatest source of radiation", will be stored "for some time", then sent back to the Soviet Union for reprocessing.

The remaining waste (liquids with a "high radiation content" and "discarded installations and parts") will be sent to the "isotope cemetery" established in a lenticular clay deposit near Puesto-oksizilagy, already used for radioactive wastes from industry and medicine.

For the Energy Ministry, Deputy Minister Gyula Szeker spoke of current fears regarding nuclear power, stressing that the western nuclear debate was encouraged by political, commercial and other interests which used "the revulsion felt for nuclear weapons" to "create a mood of opposition".

What Szeker did not mention was that the plans for the Paks power station depart from the standard Comecon doctrine that concrete containment vessels are unnecessary, and a capitalist ploy to raise costs. However "safe" the reactor, the Hungarians, it appears, will not neglect a little extra protection.

This somewhat more realistic approach to safety was also reflected in the speech of Imre Markoja, Minister of Justice. He admitted that even when all safety requirements are met "there can be some damage". Matters of responsibility and indemnity, he said, are therefore covered by the Bill. Although damage "due to exceptional events" in the course of operation of the reactor and the transport of nuclear materials is the least likely to occur, it is, however, the most dangerous. Indemnification in the case of damage, would be guaranteed by the state.

Vera Rich

NEWS IN BRIEF

French data systems sabotaged

PROGRAMMED tapes were stolen and fires were set at computer installations in Toulouse by two anti-computer groups last week. The urban guerilla group Direct Action claimed it had removed material "destined for use by the secret services" from the Toulouse offices of Philips Data Systems which it would later release. The second attack, a fire set at the rival computer company, CII-Honeywell-Bull, was claimed in a letter to *Liberation* by a group of "computer technicians well placed to appreciate the dangers to society," calling themselves the Committee to Liquidate or Neutralise Computers. The attacks followed a French government announcement of its plans to issue tenders for extra facilities to expand telematic memory banks for industrial, official and public use.

Jailed South African physicist in court

DR Renfrew Christie, the Oxford-trained nuclear physicist made his first court appearance last week to hear charges that he gave information about South Africa's nuclear programme to banned organisations. Christie, aged 30, has been jailed and held incommunicado without access to lawyers or friends under Section 16 of South Africa's Terrorism Act since his arrest shortly after he returned to take a job at the University of Cape Town last January. He is accused of intending to pass information about the location of South Africa's secret nuclear test sites, and a plan of the layout of the nuclear power station at Koeberg, to the African National Congress and the South African Christian Institute, two banned opposition groups. The trial will continue this week.

AGR plans approved

IN a reversal of her previous decision, Prime Minister Margaret Thatcher has been persuaded to approve plans for the construction of two British made advanced gas cooled reactors as part of the UK's £10 billion nuclear energy programme.

The Central Policy Review Staff and the Department of Industry argued strongly that the British nuclear industry could not develop competitively if the Prime Minister's preference for the American-built pressurised water reactor excluded the AGR. At stake are 3,000 AGR-dependent jobs in boilermaking, pipe and valve manufacturing, and engineering. Capital costs for the two proposed plants, at Heysham in Northwest England, and Torness in Scotland, have risen by 40% to \$2.8 billion in the past year, partly as a

result of increased public demands for safety. The current reactor design has several new safety features, including an enlarged reactor diameter for easier inspection and repair, and extra fuel channels to permit the reactor to maintain full power while operating at less corrosive lower temperatures. The plans also include a spherical concrete containment vessel instead of the cylindrical one.

Fewer Britons die at work

FEWER people were killed in accidents at work in the UK in 1978, according to the Health and Safety Executive Report* for 1978/79. The reduction in deaths — 498, compared with 648 in 1974 — is attributed to a greater concern about hazards at work and better prevention resulting from the 1974 Health and Safety at Work Act.

But fatalities among coal mining and railway workers show a disturbing upward trend. Mine deaths increased from 40 in 1977 to 63 in 1978; those on the railways stood at 48 for 1978, 14 higher than the average figures for recent years.

Approximately 20% of the HSE's total expenditure of £49 million was spent on research, testing and scientific support services for the year in question says the report. Extramural funding accounted for £2.6 million, of which two thirds were devoted to safety in the nuclear industry and to occupational health. Extensive research into the safety of pressurised water reactors and the hazards of exposure to plutonium were studied, safety procedures for genetic engineering were developed and the possible toxic or carcinogenic effects of a number of substances encountered in the workplace were investigated.

*Health and Safety Commission Report 1978/79. Available from HMSO £1.75.

UMIST moves into biotechnology

THE University of Manchester Institute of Science and Technology (UMIST), in a move to become involved in biotechnology, has appointed its first Professor of Applied Molecular Biology. Dr Paul Broda, whose expertise is in the field of bacterial plasmids, will take up the post in the revamped Biochemistry Department of UMIST this September.

UMIST plans to back up the appointment of Dr Broda with a number of other new posts so as to assemble a team that will carry out fundamental research in areas of molecular biology that have potential industrial applications. One particular area of interest will be in antibiotic production from the streptomycetes. Another will be in the use of bacteria to derive end products of commercial value from lignin, a major waste product of the pulp industry.

Report says 55,000 UK smokers die each year

ON World Health Day last week a report issued under the auspices of the World Health Organisation warned of the risks of smoking, and singled out the UK as the worst sufferer. Some 55,000 there die prematurely each year as a result of smoking, says the report, compared with an over 18 annual death rate from all causes of 650,000. Some 41% of adult Britons are regular smokers; one in ten of these will die prematurely of causes directly attributable to smoking and one in four from diseases in which smoking is a major factor.

A study published last week by *Mintel*, a market research magazine, showed that while the number of cigarettes smoked in Britain fell slightly, from 125.9 billion, to 125.7 billion, from 1977 to 1978, the weight of cigarette tobacco smoked rose 10% from 197 million lb to 218 million lb. *Mintel* attributes this to the increasing proportion of sales taken by king-sized brands (10% in 1975, 50% in 1978 and a projected 70% in 1981). This rise in turn is attributed to the adoption in 1978 of EEC-wide tax rules, which favour larger cigarettes.

Academy recommends cut in formaldehyde exposure

CONSUMER exposure to formaldehyde gas, in particular that resulting from the use of urea formaldehyde (UF) foam for house insulation, should be reduced to the "lowest practical concentration" to avoid its irritant effects, according to a panel of the National Academy of Science.

In a report prepared for the US Consumer Protection Commission, the Academy's Committee on Toxicology refers to two European studies, one carried out in West Germany and the other in Denmark, as evidence that "there is no population threshold for the irritant effects of formaldehyde in humans."

The CPSC says that it has received more than 600 complaints from consumers living in homes insulated with UF foam. Reported symptoms range from eye and skin irritation to breathing difficulties, persistent nosebleeds, and nausea.

The Commission adds that the Academy report will help it to develop regulatory standards for consumer products containing formaldehyde. Both the commission and the formaldehyde industry are particularly concerned about the results of recent tests on laboratory rats carried out by the Chemical Industry Institute of Toxicology, which, although not yet completed, already show that 20 per cent of rats exposed to formaldehyde have developed an unusual form of nasal cancer.

FEATURES

Let there be light!

The recent growth of conservative religion in the US has injected new vigour into attacks on the teaching of evolution. **David Dickson** reports from Atlanta, Georgia

MORE than a hundred years after the publication of Charles Darwin's *Origin of Species*, it comes as a surprise to discover that almost half the adult population of the US believes itself to be directly descended from Adam and Eve.

Yet fifty years after a Tennessee courtroom witnessed the public ridiculing of divine creation, creationist beliefs remain as strongly held as ever.

The debating skills of Clarence Darrow in the famous Scopes 'monkey' trial of 1925 may have temporarily taken the wind out of the creationist sails. But today both supporters and critics agree that the creationist movement is growing in both strength and confidence buoyed by a rising tide of conservative ideology that is rapidly becoming a powerful force in American politics.

Many schools throughout the country, for example, are now required to teach creationist beliefs in parallel with evolutionary theory. And in at least six states — including Illinois, Florida, and New York State — legislatures are discussing bills which would make such practices compulsory.

Emotions on the issue run high. Most scientists continue to treat the creationist movement with derision and scorn. "These people are using glib salesmanship to sell an academic snake-oil for the general population" says Dr William Mayer, Director of the Biological Sciences Curriculum Study in Boulder, Colorado.

In the south and mid-west, however, the reaction is different. Here support for an absolute morality has strong appeal, basing itself on a literal interpretation of the Bible.

These regions have been fertile ground for creationist organisations which, in the words of Dr Henry Morris, director of the California-based Institute for Creation Research, seek to "reverse the dangerous drift of our country and its educational system into humanism, socialism, amorism and atheism".

Nowhere has the debate been fiercer than in Atlanta, Georgia. Last month the state legislature narrowly failed to approve a bill requiring that, wherever evolution is taught as part of a biology course in state schools, equal time should be given to creationist theories. Passed by the Senate,



God creates light: a creationist's view of evolution

the bill failed in the House of Representatives in the closing minutes of the last legislative session.

The state Department of Education had argued strongly against the bill, largely on the grounds that details of the curriculum should be left to local school boards. And among other critics the bill attracted the attention of the national 'atheist' organisation which had been responsible for having school prayers declared in violation of the Constitution.

The explicit involvement of the atheists has been a red rag to conservatives such as Judge Braswell Dean, one of the leading supporters of the creationist movement in Atlanta, who has accused the organisation of defending the 'monkey mythology of Darwin'.

To Judge Dean — and other creationists — evolution is an "animal fairy tale", based largely on a "superstitious" trust in chance in its belief that random mutations could have produced the current diversity of animal types. "Scientific creationism is far more scientific and less religious" says

the Judge, who blames the teaching of "humanistic" values in schools for social problems from rising crime rates to abortion, pornography and pollution.

Not all creationists are as strident. But they do share a common belief that the teaching of evolution implies a relativity in social values that undermines both traditional codes of morality in general, and the authority of the Bible in particular.

So far the creationists have had little success in convincing the courts of their case. In most instances the barrier has been the constitutional requirement that the state should not teach religious principles. These were the grounds, for example, on which the courts declared as unconstitutional an 'equal time' bill passed in Tennessee in 1973.

Undaunted by such setbacks, creationists are now trying to get round this problem in two ways. Firstly they argue that, since it is impossible to produce scientific 'proof' of evolution, it is no more than a hypothesis which might otherwise be described as a faith or religion.

Secondly, the creationists seek to convince the legislators that creationism can be legitimately called a scientific theory. And they distinguish what is now called 'scientific' creationism from both 'divine' creationism (responsible for the failure of the Tennessee bill) and even from 'biblical' creationism (ridiculed in the Scopes trial).

To defend this position, scientifically-trained creationists now scan the scientific literature for uncertainties, ambiguities and potential errors in the ideas of conventional evolutionists.

Gaps in fossil records and uncertainties in dating techniques, for example, are used to weaken confidence in what is called the 'evolution model'. And where science has disproved rigid immutability by experiment, creationists will now allow 'adaptive mutations' — but still deny that one species can evolve into another.

Most scientists reply that weaknesses in a rigid interpretation of evolutionary theory do not undermine their general confidence in the principles involved; and they accuse creationists of distorting the scientific method, and cause-effect mechanisms of scientific explanation, into a form which few laboratory workers would recognise.

Creationists see it differently. "Negative evidence against evolution is the same as positive evidence for creation", says Dr Morris.

Whatever the philosophical disagreements, few deny that creationism has found a ready audience outside the scientific community. In particular school curricula remain a major battleground for the creationists and their opponents, largely because these are expected to demonstrate compatibility with community values.

A typical case is Cobb County, a suburb of Georgia. Last December biology courses were removed from high school graduation requirements in an attempt to resolve a conflict originating 18 months earlier when the local school board passed a resolution requiring evolution and creation to be given 'balanced treatment' in school courses.

Heated objections from science teachers and some community leaders led to eventual agreement that a voluntary course on 'comparative theories or origins' would be offered for high school students and that Darwinian evolution would not be included in any school biology course.

The Cobb County dispute is typical of conflicts now being fought out across the country. Most have in common the fact that, as Dr Dorothy Nelkin of Cornell University points out in her book *The Science Text-book Controversy*, the loudest critics of evolution are not from lower-class, uneducated backgrounds, but tend to be middle-class, technically-trained citizens.

Cobb County, an area of rapid post-war growth centred on the communications and aerospace industries, typifies what

Nelkin refers to as the 'paradox' that fundamental beliefs tend to flourish in those parts of the country which have recently become centres of high technology industries (for example Southern California and Texas).

Also typical of the Cobb County controversy is that it has taken place against sharp religious differences in the local community. Such differences have traditionally polarised around conflicting ways of interpreting the Bible. Indeed rather than a conflict between religion and science, the current disputes centre around the legitimacy of different forms of knowledge, whether used to support religious or scientific ideas.

In Atlanta, strong support for creationism has come from the more militantly conservative churches belonging to the Southern Baptist Convention, members of the currently dominant group within which is now the largest protestant sect in the US. In contrast, more moderate theologians are among the most persistent critics of biblical literalism, and have been

'Evolution is an animal fairy tale' — Judge Dean

among the most keen to keep creationism out of schools.

Further controversy has focused on the role of the Institute for Creation Research, (ICR) and was ignited when it was discovered that the 1978 resolution passed by the school board was almost identically worded to a model resolution circulated by the institute.

ICR is a division of the Christian Heritage College, a group which split from the more liberal American Scientific Affiliation in the 1950s largely over conflicting views on whether the Bible should be taken literally or metaphorically.

Since then, the institute has become a powerful centre for the teaching and propagation of creationist ideas which like other modern evangelical organisations, it has done with spirited fervour.

Institute members were also influential in setting up in 1963 the Creation Research Society, an organisation consisting of over 600 individuals with postgraduate degrees in scientific or technical subjects concerned with developing a 'scientific' critique of evolutionary theory — and arguing the case for a 'scientific' alternative.

Although denying any orchestration of local creationist movements, Dr Morris admits that an important role of ICR is to provide model resolutions and educational literature to groups prepared to support the contention that "teaching evolutionary theory alone is contrary to academic freedom, civil rights, and the freedom of religion".

Ironically much of the creationists' critique of the values implicit in evolutionary theory are similar to those coming from a very different perspective

which identify Darwin's ideas with the political norms of Victorian capitalism. But despite apparent similarities, the antagonisms are sharp.

Professor Richard Lewontin of Harvard University, for example, describes the recent rise of creationism as reflecting a "wave of anti-scientism", suggesting that its rejuvenation is "because intellectuals are identified as the allies of social movements" that challenge traditional power relations in society.

Conversely Dr Morris points out that many of the more politically radical evolutionists are among those questioning the more dogmatic aspects of Darwinian theory. But he castigates them for doing so "with prejudices tied to Karl Marx rather than Adam Smith".

It is difficult to judge the impact of the creationist movement. Some argue that, by causing publishers and teachers to drag their feet over introducing evolution into the class-room, creationists — in the words of one educator — "have had a tremendous impact on students in denying them access to scientific knowledge".

Others, however, are more prepared to be flexible. They use arguments from the sociology of knowledge to defend the interpretation of science as a belief system; and side with the creationists in their criticism of the more dogmatic assertions of some scientists and textbook writers.

Of greater concern to educationists are the implications of a movement which appears to attack abortion and homosexuality and defend the 'free world' with the same degree of dogmatism that it criticises in others.

Jeremy Rifkin, author of *The Emerging Order*, warns that what is now a religious movement, reacting in part to the 'idolatry' of scientific and technological truths, could turn into the opposite. "Christian doctrine, made an adjunct to right-wing and capitalist policies, could provide the necessary self-imposed order that a fascist movement in America would require to maintain control over the country during a period of long-range economic decline" he writes.

Creationists are used to responding in kind. Dr Morris argues that both fascism and communism have their roots in evolutionary thinking. He attacks the "humanistic and socialistic" biases of textbook publishers who refuse to mention scientific creationism. And he claims that creationist ideas have unfairly come under "bigoted pressure from the liberal news media".

Whatever the words used, the bitterness of the Georgia conflicts indicate that creationist ideology is unlikely to disappear. And it seems only a matter of time before some state requires creationism to be taught wherever Darwinism is invoked and another court is asked to rule on the respective definitions of science and religion. As Dr Mayer says, "It is all part and parcel of the signs of the times". □

Patenting nature's secrets and protecting microbiologists' interests

THE patenting of inventions in microbiology, is arousing unusual interest the United States and Europe. **Stephen Crespi**, Patents Controller at the UK National Research Development Corporation examines recent developments in Europe

THE US Supreme Court is hearing the appeal brought by Dr A Chakrabarty in 1972 over the rejection by the Patent Office of an attempt by GEC to patent an oil-consuming strain of *Pseudomonas* obtained through genetic manipulation. And in Europe, the European Patent Office has recently changed the mechanism, called Rule 28, for ensuring public availability to third parties of new strains of microorganism deposited in culture collections for patent purposes.

The legal controversy aroused in the US by the long-running Chakrabarty case has not fully erupted in Europe because there has been no comparable test case. But Europe must view developments in the US with interest because in a world in which legal systems borrow from one another the outcome in the US may affect that in Europe.

Some of the older American decisions show judicial condemnation of attempts to patent nature's secrets where living organisms are involved — although patents were allowed for meritorious discoveries of inanimate products of nature, vitamin B₁₂ being one of the most celebrated examples.

In Chakrabarty the fundamental point is simple; is a living organism which otherwise complies with legal requirements for patentability nevertheless disqualified because it is alive? In answering this question the court has to consider how to accommodate the product of genetic engineering in a patent system based on the models of classical physics and chemistry. However, if the decision of the Supreme Court later this year is limited to man-made organisms which are the result of genetic engineering it will be disappointing after so much effort has gone into dealing with the broader issue.

To put these developments into perspective it is worthwhile summarising the possible categories of patentable invention in microbiology. Microbiological processes have long been recognised by courts as suitable for process patents; and the newer patent statutes in Europe specifically refer to them. Patent claims may be presented for inventive developments of any of the methods of microbiology which serve a useful economic purpose such as improvements in culture media, culture conditions and the choice of strain used. Processes in which the sole novelty lies in the use of particular strains,

especially newly developed strains, have become conventional subjects of "process-of-use" patents.

Products produced by microorganisms such as antibiotics and enzymes have for a long time been patentable in the form of product claims where the products were novel and where the patent law of the country concerned permitted the so-called product-*per-se* claim, i.e. not limited in scope to a particular process. Where the product was not new, and novelty resided only in the process of making it, it was customary to use both process claims and also, where possible, the product-by-process claim, i.e. a claim to the product when made by the particular process. However, it is with the other type of possible product claim, the claim to the new strain of microbial cells themselves, that tension has arisen between applicants and examiners. Claiming the microbial biomass as a useful end product was generally acceptable but claims directed to the new strain *per se* seem more recently to have raised the kind of philosophical objections in the Chakrabarty case.

The traditional attitude of the UK patent system towards patent protection involving the use of living matter has been cautious but attentive to the needs of industry and applicants for patents. The following principles have operated: the concept of "manufacture" as essential for patentability; the fact that many living substances can be bought and sold like other commodities; giving the benefit of the doubt to an applicant where the law was uncertain but where the decision could be tested by a higher authority if any contestant so wished. The test of "manufacture" was whether the product was itself a manufacture or could be applied to manufacture, or whether the process was technical in nature as distinct from the establishment of conditions under which the organism was developed by essentially biological laws. Long before the subject attracted public comment, yeast manufacturers took out patents for new yeast strains, presumably because the alternative of trade secrecy was non-existent where the live microorganism was itself the item of commerce. British patents were also granted over the past twenty years for other new strains, cell lines, and attenuated viruses intended for vaccines. The *Fusarium graminearum* strains

intended as sources of single cell protein were patented without challenge in the UK in 1974 although held unpatentable in Eire and Australia by official ruling a few years later.

The British Patent Act of 1977 was brought in line with the European patent law of 1978 and practice should run in parallel under both systems. Recent informal discussions with EPO officials suggest that product-by-process claims to microorganisms will be accepted and that the decision on the patentability of unrestricted product claims to the strain *per se* will be taken soon. Much may depend on the circumstances of each case.

The number of patent applications on genetic engineering procedures reaching the publication stage is increasing. Once Patent Office Examiners have fathomed the extraordinary complexity of the subject there should be a spate of patents granted for techniques. It is difficult to see why recombinant DNA plasmids should be treated any differently from other chemical substances but whether claims to plasmids and transformed strains are obtained remains to be seen. What commercial value do these claims have and can they be policed inside the competitor's factory gates? We cannot yet judge the value of these to the innovators especially with so little experience of their usefulness. Therefore instead of being negative or restrictive we should explore ways in which the patent law can encourage these new areas of research.

What is the objection to patenting living matter? Some condemn it by asking where it will end and they argue that a logical extension to higher life forms supports their view. But law and logic are not identical and this argument has to overcome two objections.

The first is that one class of higher life forms is already protected, namely plants by the plant patents in the US and plant breeders' rights elsewhere. The latter are distinct from patent protection and show an interesting difference: the plant breeder can control not only the commercial marketing of the reproductive material of the new variety but also its subsequent multiplication whereas normally once a patentee has sold the product covered by the patent he cannot control it further. Secondly, patent legislation in some countries (including that of the European Patent Convention) specifically excludes patents for plant and animal varieties.

The patent law exists to benefit research and its financial supporters. If it becomes necessary to draw an arbitrary line between what living organisms are or are not patentable it is within the wisdom of judges

and administrators of the law to do so in a way which supports technology and is generous to the inventor without harm to anyone else. Borderline cases will arise where the meaning of the terms 'plant' and 'animal' will have to be looked at closely but the constraints against patents for higher life forms are clearly built in to the present laws, at least according to the European model.

In presenting their survey of the patent law to the Supreme Court, the US judges of the lower court have approached the question on the pragmatic ground of usefulness to industry where they see no distinction between living microorganisms and chemical elements and compounds. If it is socially acceptable and desirable for pharmaceutical companies to develop new microorganisms and produce products containing living material such as "live" virus vaccines there can be no reason for restricting the patent cover available for these innovations.

To erect obstacles to patent protection is to encourage the secrecy which the patent law is designed to discourage. The patent system has in recent years become much more open especially with its emphasis on early compulsory publication of patent applications which in the past often remained confidential in the Patent Office for a long time. However, the emphasis on early publication has caused problems for microbiologists because a new strain of a microorganism must be available to third parties at the same time as publication. The European Patent Convention of 1973 set the trend on this point.

One of its regulations, Rule 28, had said that a new strain must be deposited in a culture collection before a European patent application could be filed properly and also insisted on the accessibility of the strain to others, subject to a few conditions, on publication of the application 18 months or so after the priority date. This contrasted with US and Japanese patent law where release of the strain is obligatory only when an enforceable right is obtained.

However, the European Patent Office has recently modified Rule 28. Availability of the strain to third parties can now be restricted between first publication of the application and the eventual grant of patent rights. During this time the applicant will be able to limit access to the strain to an independent expert acting on behalf of third parties but bound by certain conditions including that of not passing the strain out of his hands.

This improvement of the rule concludes over six years of effort by European industry and others to persuade the authorities that unrestricted availability of the culture before any rights are granted involves loss of control at too early a stage. This has been one of the first controversial questions tackled and solved by the EPO since it began operation in June 1978. The decision to change the rule has anticipated

Evolving ideas

EVOLUTION is not really in trouble, of course, it has never been healthier. It has gone into the computers. This is a sad business when one thinks of the halcyon days about a century ago. It is true that Darwin, the brooding sage, was a recluse at Down, but his supporters were having a wonderful time. We have been reminded of this in the splendid television series "The Voyage of the Beagle", and especially by the exciting re-enactment, on this programme, of the confrontation between Bishop Wilberforce and Thomas Huxley. The question asked by the Bishop was on which side Huxley claimed descent from the apes. Today we might reply that the maternal line of inheritance has a slight edge because mitochondria probably travel with ova. However, Huxley's thunderous response was directed personally at Wilberforce, whereupon it is said, a young woman fainted. Ironically, Queen Victoria, the supreme head of Wilberforce's church, carried a mutant gene for one of the blood-clotting globulins (Factor VIII), for the male haemophiliacs among her descendants provided a tragic and classic example of the ruthless effects of natural selection.

Evolution then set forth for many years on an adventure among fossils of extinct animals and plants. Pterodactyl, *Tyrannosaurus* and *Archaeopteryx* became household words. Descriptions of the bones of our ancestors were often in the news. The Scopes trial put evolution into the field of entertainment. Next, biochemists devised methods for determining the sequences of amino acids in proteins. It became possible to measure evolutionary divergence numerically in terms of amino acid differences between similar proteins in various species. Haemoglobins of gorillas, chimpanzees and human beings were distressingly similar, but widening differences were found in other species. The fun was disappearing from evolution, but worse was to come for the classical taxonomists. Incredibly rapid new methods were perfected for measuring long sequences of nucleotides in DNA, and the results, photoreduced to near-illegibility, appear in *Nature* almost every week.

A few years ago, we could examine

the UK report on biotechnology published last week (see *Nature*, 10 April, page 502) which strongly criticised the lack of protection under Rule 28.

Rule 28 was also updated to conform to the corresponding rule in the Budapest Convention of 1977 which provides that deposition of the strain in a single officially recognised culture collection will suffice for the individual, national procedures.

The detailed application of the independent expert idea remains to be



THOMAS H. JUKES

phenotypes but we never expected to be able to read genes. Now every sequencer can become a computer-aided evolutionist. Viruses evolve just like entire organisms; in fact, genes in simian virus 40 and the polyoma virus have diverged even further from each other than genes for the alpha and beta chains of haemoglobin, which have spread far apart in the 500 million years since they separated from a common ancestral molecule. But who knows how fast viruses evolve? They leave no fossilized imprints in the rocks as guideposts of their age.

To look for a gene in DNA, you scan for "open reading frames". These are regions in nucleotide sequences that are free from occurrences of TAA, TAG and TGA: the "stop signals" in protein synthesis. It is becoming quicker to find new proteins in DNA sequences than to separate them from protoplasm. It is even possible to find genes that are no longer in use. Phil Leder recently called such a gene (for a mouse alpha haemoglobin chain) a "rusting hulk", because it had accumulated so many changes, including deletions and insertions.

All evolutionary changes result from inherited changes in DNA molecules. It is difficult to get emotional about alterations in the linear arrangement of A, C, G and T. The spiritual descendants of Bishop Wilberforce must find it rather dull to argue with computer programs.

worked out but the intention is that the expert will carry out experiments on behalf of third parties and potential opposers of the patent to test the patent disclosure and make an evaluation of the invention. The expert will be chosen by agreement between the applicant and the party requesting the strain or from an official list of recognised experts. Consequently the services of eminent microbiologists will be in demand and experts who might be willing to undertake this role are being canvassed. □

CORRESPONDENCE

Nuclear technology is not the threat

SIR,—Mr A. B. Lovins' article (28 February, page 817) confines itself almost entirely to a discussion of the use of reactor grade plutonium in the construction of bombs, either by terrorist groups, or by governments. I do not believe myself to be enough of an expert on these esoteric branches of industrial chemistry and nuclear physics to comment usefully on the technical aspects of the article, and in any case I would prefer to concentrate on some implicit non-technicalities.

First, there is the claim, not made here by Mr Lovins, that the increased availability of dangerous materials will increase the risk of their misuse. While there is some truth in this depressing doctrine, I feel that the size of such risks is dominated by the proportion of people who are keen to use terrorist methods, rather than by the technology of their particular community.

No, the question surely is whether we are to give up clean, reliable, safe, economical and enormously useful techniques simply for fear of what the malevolent may do. If we are, where does this line of argument stop? Do the Friends of the Earth want us to give up aviation, liquid natural gas, or genetic engineering? And if not, why not? Or, to put it another way, should Adam have stopped Eve from eating that Fruit?

In addition, I have some specific quarrels with Mr Lovins' article. Who, for instance, were the "three high-technology nations" whose ministers were so misled? Again, no-one associated with the anti-nuclear movement is entitled to complain about "lost, oversimplified or garbled" advice. And what on Earth does he mean by saying that "the implication that the effects of even a crude, minimal 0.1-1 kton explosion would be tolerable for a free society is at best disingenuous"? Non-free societies are entitled to proceed, apparently.

Without making any statement about the possible carnage in such an explosion, I would like to point out that conventional accidents sometimes take thousands of lives in free societies, and presumably in non-free ones too. The multiple standards of response to such disasters is sadly but glaringly highlighted by the failure of the Morbi dam in Gujrat State, India, in August. It seems to have taken some hundreds of lives at least, and passed almost unnoticed in the Year of Three Mile Island.

Yours faithfully,

J. F. CRAWFORD

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Comparing the diets of laboratory animals

SIR,—It has been demonstrated several times that toxicological experiments performed in different laboratories are liable to unexplained variations in result. Amongst the possible reasons for this observation, are differences in diet of the experimental animals (see for example Hathcock and Coon *Nutrition and Drug Interrelations*, Academic Press, New York 1978). Of 89 randomly-chosen recent publications in the fields of oncology, pharmacology and toxicology, more than half failed to specify the diet fed to their laboratory rodents. Often considerable portions of the 'methods' section were description of the animals, sources of

chemicals used, etc, but the nearest any of the authors got to specifying their diets was to give the manufacturer's name. This implies that the authors consider stock diets to be standard. However, my survey of 24 UK stock diets for mice and rats has shown that for each nutrient, very large differences in the diet composition exist. Amongst nine minerals, the coefficient of variation ranged from 16% to 80%, with a median value of 28%. Similarly for 12 vitamins, the range was from 20% to 91%, with a median of 62%.

The survey was based on the best information available, but in all cases it is a calculated figure only, based on the ingredients of the diets. Only one British manufacturer has been prepared to supply details for one diet of average analyses after production. Some manufacturers at extra cost will provide analytical details to customers on a batch basis, and it is these figures that are required for a scientific evaluation of diets on a comparative basis. The majority of nutritionists use semi-synthetic diets, rather than stock diets, and it is perhaps for this reason that their attention has not been focused on diets used by non-nutritionists and why so little nutritional work has been done in this field.

It is the intention of the Laboratory Animals Centre to try to obtain information about actual analyses, for nutrients and contaminants, on stock diets worldwide. It would then be possible to help those who are trying to explain differences in results between similar experiments in different laboratories, in which it is suspected that dietary variation might have been important. In the furtherance of this aim, it is suggested that diet manufacturers and scientists who have relevant analytical data should contact the Laboratory Animals Centre to discuss the possibility of collaboration in such a study.

Yours faithfully,

A. WISE

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Establishing the case for the Maunder Minimum

SIR,—The News and Views contribution of 31 January (page 427) entitled "Was there a Maunder Minimum?" projects a superficial understanding of the meticulous work by solar astronomers, historians, climatologists, palaeobiologists, and scientists from a host of other research disciplines supporting a marked diminution in solar activity during the 17th century.

Table 1 of the article shows that there were nine naked eye sunspot records during the decade beginning 1610, six in the 1620s, nine in 1639, two in the 1640s, three in the 1650s, one in the subsequent decade, then none, and finally one in the 1680s. Taken at face value, the new *fang chih* records would seem to lend support to a dramatic reduction from about 1640 in the number of sunspot groups or individual spots large enough to be seen by the naked eye under suitable atmospheric conditions. But a direct interpretation of such limited data is superficial. As your article stresses, the *fang chih* do not present an unbiased sample of data, any more so than the records in the official dynastic histories. At least for the latter one has some check on completeness of the data by looking at the continuity of official records of other astronomical phenomena. However, because

of political, social and astrological influences, oriental sunspot records are by themselves of little value; as previous investigators have been careful to stress, they provide at best merely circumstantial evidence for excursions in solar activity. There is no point arguing for or against a Maunder Minimum on the appearance or absence of naked-eye sunspot records from the orient when post-telescopic (1610) occidental records, free from political-astrological influences, are available and when a wealth of other indicators of solar variability have been investigated. Yet none of these are mentioned, the reality of the Maunder Minimum being called into question on the basis of a few provincial oriental records of doubtful reliability.

The leading late 17th century observational astronomers lamented the scarcity of sunspot activity. Here we have more than just a chance mention of detections, but professional observers insisting that it had been a decade or more since any spots had been seen. Spots there certainly were during the Maunder Minimum (1645-1715) but greatly reduced in frequency and extent. Thus, for example, Stephen Gray noted in 1705 "the Sun was much more productive of (spots) in (Galileo's) time than it has yet been in ours, and the Regions Producing them had a far Greater extent". However, if one was hesitant to accept the direct evidence of contemporary scientific writings, indirect indicators provide near-conclusive proof of reduced solar activity. A history of the flux of cosmic rays, related to solar activity (and hence to sunspot occurrence) can be constructed from isotopic anomalies preserved in tree rings and ice cores. All such investigations reinforce the case for the Maunder Minimum.

Yours faithfully,

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For any readers wishing to follow up the original Chinese language paper on which the *News and Views* article was based the reference is *Nanjing Daxue Xuebao* (*Ziran Kexue Ban* No. 2/31/1979 and not 1976 as printed: ED

Lead petrol additives

SIR,—In an otherwise valuable contribution to the debate on the health impacts of childhood lead exposure, M.R. Moore (24 January, page 334) may have unnecessarily compromised the case against leaded petrol by conceding significant economic benefits.

It has long been accepted that lead petrol additives are a major contributor to combustion chamber deposits responsible for increased octane rating demand. Measurements of this effect (e.g. G. Cornetti *et al*, *The Journal of Automotive Engineering*, June 1971, 8-14) have demonstrated fuel efficiency penalties that are greater than the gains made possible by increased compression ratios with high octane, leaded petrol.

Yours faithfully,

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NEWS AND VIEWS

Interferons and the immune system

from Barry R. Bloom

The current exuberance about interferon reflected in these pages referring to interferon as "potentially the wonder drug of the 80's" recalls the comment of Michael Faraday on being questioned sceptically by Gladstone on the uses of his research on electromagnetism, "One day, it will give rise to a great industry upon which you can levy taxes." Why such enthusiasm? At least part of the answer lies in the recent appreciation of the regulatory effects that interferons exert on the immune system.

Interferon has been defined by its antiviral effect and has been considered almost exclusively as a potential antiviral agent. (The work of Gresser on the other biological effects of interferons stands as an heroic exception (*Cell. Immun.* **34**, 406; 1977).) Yet evidence for its efficacy in acute virus infections has been relatively disappointing until recently although it does seem a promising treatment in hepatitis, rabies, varicella zoster, herpes keratitis and rhinovirus (common cold) infections. But the most significant boost to the budding interferon industry comes from trials of interferon in cancer patients, initially stimulated by the studies of Strander and his colleagues on osteosarcomas, and reinforced by preliminary findings in non-Hodgkins lymphomas, breast cancer and multiple myeloma. So far, however, the optimism is based on published results in fewer than 50 patients in trials that have not been randomized, blinded or well controlled. As more and cheaper interferon becomes available, larger trials will be carried out.

The principal approach to studying both

the antiviral and antitumour effects of interferon has been to look at the direct effects of interferon on appropriate tumours or virus-infected target cells. Various metabolic pathways activated by interferon have been elucidated, involving 2' 5'-polyadenylate synthetase, protein kinase and nucleases which may play a part in blocking initiation of viral or host protein synthesis and in breakdown of viral and host mRNAs (see *Nature* **282**, 364; 1979). One probable consequence of these regulatory changes is a relatively general inhibition of replication of various normal and neoplastic cells. *In vivo* experiments confirm that interferons inhibit tumour growth in several animal models, but they can also suppress liver regeneration in partially hepatectomized mice and even inhibit growth and development of suckling mice.

In the light of the general growth inhibitory effects of interferons, it is not surprising that the first effects of interferon on the immune system to be studied were its immunosuppressive activities. Several laboratories showed that interferons can inhibit antibody formation to thymus-dependent (sheep erythrocytes) and thymus-independent antigens *in vivo* and *in vitro*. In this regard type II 'immune' interferon (produced by T cells) has been found to be 10-100 times more suppressive than type I interferon (the type produced by leukocytes and fibroblasts) (Sonnenfeld *et al. Cell. Immun.* **34**, 193; 1977). Interferons suppress mitogenic responses of both T cells and B cells *in vitro*. Indeed, interferons seem to be responsible for mediating the suppression of the concanavalin A-activated human T suppressor cell (Kadish *et al. J. exp. Med.* in the press). In most studies *in vivo*,

relatively large amounts of crude interferons have been used, and while suppressive effects were observed, interferon can hardly be regarded as one of the more potent immunosuppressive agents available. Indeed, little evidence for suppression, of antibody formation at least, has been seen in patients receiving interferon therapy for cancer or virus infection. Although interferon suppresses graft-versus-host reactions in animals, its usefulness in human bone marrow grafts is limited because its growth-inhibitory activity has blocked bone marrow proliferation and reconstitution. Thus the balance between useful immunosuppression, and unacceptable side effects may be delicate.

Interferon and NK cells

In 1975 it was first recognized that normal spleen cells of some strains of mice were cytotoxic *in vitro* selectively for neoplastically transformed cells (Kieślinski *et al. Eur. J. Immunol.* **5**, 112; 1975). The cells responsible for this spontaneous cytotoxicity were termed 'natural killer' (NK) cells and are quite distinct from conventional B cells, T cells or macrophages (*Transpl. Rev.* **44**, 1979; *Natural Cell-Mediated Immunity against Tumours* (ed. Herberman) Academic Press, in the press). Athymic nude mice spleens possess increased NK activity, whereas cells from individuals with Chediak-Higashi syndrome or its murine analogue, *beige* mice, are almost totally deficient. The provenance and fate of NK cells remain unknown. NK cells bear surface markers characteristic of the T-cell lineage (Minato *et al. Ann. N. Y. Acad. Sci.* in the press), but also seem to share characteristics in common with

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macrophages (Lohmann-Matthes *et al. J. Immun.* **123**, 1883; 1979). Alternatively, they may represent an independent lymphocyte pathway. It may well be that NK activity is mediated by more than one cell type in different systems.

Interferon seems to be the key immunoregulatory signal for NK activity. Several independent studies earlier suggested that inducers of interferon *in vivo* such as poly(I):poly(C) or tilerone augmented NK activity in spleen cells (Gidlund *et al. Nature* **273**, 759; 1978; Djeu *et al. J. Immun.* **122**, 175; 1979). Surprisingly, known immunological adjuvants such as BCG or *Corynebacterium parvum*, which in some models have been shown to enhance resistance to tumours in both conventional and athymic mice also augment NK reactivity (Wolfe *et al. Nature* **252**, 584; 1976). It has now formally been established that interferon, including chemically-purified material, can markedly augment cytotoxicity of NK cells for appropriate target cells (Trinchieri *et al. J. exp. Med.* **147**, 1314; 1978; Zarling *et al. J. Immun.* **123**, 63; 1979; Minato *et al. op. cit.*).

Lymphoid cells are not only regulated by interferons but actively produce them. Type II or 'immune' interferon is produced by mitogen or antigen-stimulated T cells (Epstein in *Interferons and their Actions* (ed. Stewart) 91, CRC Press, Cleveland, 1978). Interaction of a non-B, non-T cell population of human peripheral blood cells with a majority of human tumour cell lines tested *in vitro* resulted in the production of type I leukocyte interferon, and there seemed to be a general correlation between the susceptibility of tumour cells to lysis by NK cells and the tumour cells' ability to induce interferon production by NK cells (Trinchieri *et al. J. exp. Med.* **147**, 1299; 1978). Several laboratories have shown that NK cells bind to appropriate target cells, and have used specific adsorption to targets to enrich for human NK cells and examine their morphology and characteristics (Timonen *et al. Cell. Immun.* **48**, 133; 1979; Roder *et al. J. Immun.* **121**, 2509; 1978; Jensen & Koren *J. Immun.* **123**, 1127; 1979). After the binding of a relatively homogeneous population of medium-large lymphocytes with striking cytoplasmic granules to susceptible target cells, these bound presumed NK cells rapidly become immunofluorescence-positive for interferon (Saksela *et al. Annl. N. Y. Acad. Sci.* in the press). While it has not formally been established that the interferon-producing cells are the cytolytic ones, the correlation between binding, interferon-positive immunofluorescence and cytotoxicity for the targets is striking.

Interferon could augment NK activity in two ways. (1) It might activate existing NK cells, like the activation of macrophages by lymphokines, or (2) it could trigger the differentiation of a precursor to the NK cell into an effector cell. It has recently been

shown in nude mice that both NK cells and interferon-producing cells are susceptible to anti-Ly 5 antibodies (Cantor *et al. Immun. Rev.* **44**, 3; 1979) and anti-Qa 5 antibodies (Chun *et al. J. exp. Med.* **149**, 426; 1979). When NK activity was depleted by treatment with anti-Ly 5 + complement, interferon restored it within 3 h, and the induced effector cells expressed the Ly 5 surface marker (Minato *et al. op. cit.*). In contrast, treatment with anti-Qa 5 + complement eliminated NK activity which could not be restored by addition of interferon, and hence serves as a marker for the NK precursor. Precisely analogous results were found using human cells depleted of functional NK activity by selective adsorption to target cells which could be induced to become cytolytic after interferon treatment (Saksela *et al. Scand. J. Immun.* **10**, 257; 1979). Thus activation of NK cells by virus or appropriate tumour cells initiates a positive feedback circuit mediated by interferon resulting in rapid differentiation and amplification of NK function.

Role of NK cells in resistance to tumours

What is the evidence that interferon-induced NK cells have any significant immunological role *in vivo*? The mechanism by which interferon exerts antiviral effects *in vivo* should be re-examined following recent experiments which show that although human interferon did not protect monkey cells against vaccinia virus infection *in vitro*, treatment of monkeys themselves with interferon effectively prevented the growth of locally inoculated vaccinia virus (Schellekens *et al. Nature* **278**, 742; 1979). These results strongly indicate that in this, and perhaps other systems, interferon may exert its protective effects not by acting directly on target cells but perhaps through its effects on the immune system. There is evidence for a significant role of interferon in natural resistance to acute infections (Gresser *et al. J. exp. Med.* **144**, 1305; 1976). Surprisingly, the interferon-NK cell system may be far more important in resistance to persistent virus infections and tumour cells.

As few as 10–100 HeLa or BHK cells produce tumours in all athymic *nude* mice; yet 10^6 – 10^7 of the same cells persistently infected with any of six RNA viruses fail to grow as tumours (Minato *et al. J. exp. Med.* **149**, 1117; 1979). The persistently-infected tumour cells were killed by NK cells *in vitro*, whereas the uninfected parental tumour cells were not. One variant of BHK cells persistently infected with VSV was an excellent inducer of interferon and capable *in vivo* of augmenting cytotoxicity to irrelevant target cells, but was itself resistant to NK killing *in vitro*. This variant formed disseminated tumours and metastases in *nude* mice.

Important evidence for a role for NK cells in restriction of metastases has

recently been provided by remarkable experiments in which a variant of the B16 murine melanoma tumour line which is susceptible to killing by NK cells *in vitro* showed enhanced growth, faster induction time and markedly increased metastatic capability in *beige* mice, which are deficient in NK cells, compared with control mice (Talmadge *et al.* this issue of *Nature*, page 622; see also Karre *et al.* this issue of *Nature*, page 624, on the possible role of NK cells in resistance to syngeneic leukaemias in mice). Talmadge *et al.* find that induction of interferon by infection with LCM virus decreased the tumour growth rate and metastatic frequency even in *beige* mice. Treatment of *nude* mice, which ordinarily reject tumour cells persistently infected with virus, with antibodies to mouse interferon not only results in the growth of the tumours, but in their generalized metastasis (Reid, *et al. 3rd Intl Workshop on Nude Mice* in the press).

Much less is known about the role of the interferon-NK system in human disease. In diseases possibly caused by persistent viruses such as multiple sclerosis and lupus erythematosus, diminished production of leukocyte interferon and NK activity *in vitro* by peripheral lymphocytes in some patients suggest that such deficiencies could predispose to persistent virus infection (Minato *et al. op. cit.*). Several investigators are finding diminished NK activity in patients with tumours (Hersey *et al. Brit. J. Cancer* **40**, 113; 1979; Pross & Baines in *Natural Cell-Mediated Immunity against Tumours* (ed. Herberman) Academic Press, in the press). Diminished NK activity was found in familial melanoma patients and their relatives suggesting that the deficiencies in these patients may be a predisposing factor to their disease, rather than simply the result of disseminated metastases.

Questions for the future

The direct biological activity and immunoregulatory activity of interferon has raised high expectations and many questions. Yet, there are very few cases in which it has been possible to disentangle the direct effects of interferons *in vivo* on tumour cells and target cells for viruses from their effects on the immune system, and this will probably require analysis of the genetic make-up of peculiar mouse strains, for example *beige* and *nude* mice.

If a positive feedback circuit regulates the differentiation of NK cells and precursors, are there factors which normally restrict or regulate this process? One wonders particularly whether T cells, or a subset, intervene to restrict the development of NK cells. Do leukocyte and 'immune' interferons have any unique functions or target activities?

Are NK cells clonal, and do they have specificity in the immunological sense, or merely selectivity? If the latter, what are the common surface determinants which they recognize in various tumour and virus-

infected cells; if the former, can these cells be cloned and specifically amplified *in vitro*?

Is the role of interferon in natural resistance to infection or tumour spread primarily local? Can systemic administration provide an adequate effect on regional responses?

Does a deficiency either in interferon production or NK activity predispose to persistent virus infections or metastases, and can *in vitro* tests of these parameters be used as diagnostic adjuncts? In patients with these diseases does interferon augment NK activity in some patients and not others, and if so, can prescreening of patients scheduled for interferon therapy for *in vitro* sensitivity to interferon identify those showing better clinical response *in vivo*? Answers to many of these questions

are bound to influence a great deal more than the prosperity of the interferon industry.

In understanding immunological effects of interferon, it is important to note that there are very few immunology laboratories that are able to produce and purify the various types of interferon and at the same time pursue their effects on the immune system. For rapid progress, immunologists will be dependent on the good will and collaboration of the interferon experts providing them with purified material for these studies.

At a minimum, one may hope that the immunologists could repay them both by producing monoclonal antibodies against various interferons and providing insights into the mechanisms by which their product exerts its effect. □

abundance of interstellar H_2O can be deduced. Here is where the new line discovered by Waters *et al.* comes in. In the Orion molecular cloud where the new line is seen, lines of most other molecules have a characteristic emission profile (a sharp spike superimposed upon a broad pedestal) indicative of rapid mass motions within a small energetic core surrounded by a larger more quiescent region. Molecules known to be masering in Orion (OH, 22 GHz H_2O , CH_3OH) do not show this profile, but non-masering molecules do. Although they are unable to resolve the emission region spatially with their 91 cm telescope, Waters *et al.* find that the 183 GHz water emission emulates quite well the spike-plateau profile of other molecules, and therefore conclude that this water transition is reasonably normally excited. This spike feature apparently arises in the quiescent region whose size (about 4 arc min) and temperature (about 80 K) are well determined from the lines of many other molecules. If the 183 GHz line of H_2O is thermalized at this temperature, Waters *et al.* estimate the H_2O abundance at a few times 10^{17} cm^{-2} . If one calculates the excitation produced by known infrared radiation fields and by collisions with other molecules, then H_2O abundances as large as 10^{18} cm^{-2} can result. The net abundance uncertainty (a factor of 10) is in fact no greater than applies to many other molecular species observed from the ground, and will be greatly reduced by use of a larger airborne telescope which resolves the source. This first result already yields important ratios for the interstellar chemist: the fractional abundance of H_2O is about 10^{-6} , consistent with ion-molecule chemical models. From ground-based studies of HDO, one deduces that $\text{HDO}/\text{H}_2\text{O} \gg \text{D}/\text{H}$, showing that water, like several other interstellar molecules, concentrates deuterium with great efficiency.

Even more recent detections of hitherto inaccessible interstellar lines and molecules have been made from above the troposphere by T. L. Phillips and colleagues, using the KAO (IAU Symposium 87, Mt. Tremblant, Quebec, August 1979). The $4_{14-3_{21}}$ transition of H_2O at 380 GHz has a spectral profile in the Orion source similar to that of the 183 GHz line, and thus is consistent with the picture discussed by Waters *et al.* Carbon monoxide, the most important tracer of the dense molecular interstellar gas, is now much better understood thanks to the detections in mid-1979 of the $J=3-2$ and $4-3$ transitions at 345 and 461 GHz respectively. These higher-lying CO transitions often exhibit marked self-reversals in their profiles, which are usually not evident in the lower-lying lines previously studied from the ground, and which are indicative of complex temperature, velocity, and density variations within the clouds. Such self-reversals may also bear on the classical

Interstellar spectroscopy

from B.E. Turner

ALMOST a dozen years ago the first interstellar polyatomic molecules were detected by ground-based radio astronomy in the microwave region. Today, the list of known interstellar molecules stands at 53 and includes 90 different isotopically substituted species. These molecules have provided a new and unique tool for studying the dense, cool component of the interstellar medium, a component now recognized as comprising half of the interstellar medium by mass. Molecular clouds which range up to 10^7 solar masses are the sites of star formation throughout the Galaxy and have an important, if not dominant, effect on the dynamics and kinematics of both the interstellar medium and of the stellar populations immersed in it.

Both the physical conditions in these interstellar clouds, and their chemistry, have been subjects of intense study over the past decade. The molecular species identified range in complexity from 2 to 11 atoms. Not surprisingly, the more complex the molecule, the more speculative our understanding about how it is formed. Thus, meaningful tests of the current theories about chemical processes in interstellar molecular clouds necessarily involve the simpler of the known species. To be useful either as tests of chemical models or as probes of physical conditions, these simple species must have reliably determined abundances.

Adequate study of important interstellar molecules is hampered or prevented by the Earth's atmosphere as it absorbs strongly in several bands of O_2 and H_2O at wavelengths longer than 800 μm , and fairly continuously in a myriad of water transitions over the entire sub-millimetre region below 800 μm . The most important

interstellar molecules are light, and have many important transitions in the sub-millimetre region, observations of which are needed to determine abundances. Among these are CO and HD, which along with atomic carbon provide the principal cooling transitions of dense clouds. Also included are all of the basic hydrides of the hydrocarbon, nitride, and hydroxyl families and of a number of elements (Mg, P, Na, Cl, Al, Ca, Fe) not yet identified in interstellar molecules. Key transitions of interstellar species such as H_2O and O_2 are of course absorbed by the corresponding telluric lines.

In the past few years a strong effort has been mounted to study extraterrestrial lines from above the troposphere. These studies conducted with the Kuiper Airborne Observatory (operated by NASA Ames Research Center) have begun to pay handsome returns with the airborne detections of the important interstellar line of H_2O at 183 GHz, two sub-millimetre lines of CO and one of H_2O , and, very recently, the 492 GHz fine-structure transition of atomic carbon.

In a series of observations from the KAO beginning in late 1976 J. W. Waters and colleagues (*Astrophys. J.* **235**, 57; 1980) made the first detection of an interstellar molecular line inaccessible from the ground, the $3_{13-2_{20}}$ transition of H_2O at 183 GHz. Interstellar H_2O has long been observed from the ground via its $6_{16-5_{23}}$ transition at 22.2 GHz. In normally excited H_2O such as occurs in the Earth's atmosphere, the 22 GHz line is very weak, thus explaining the modest (a few percent) absorption by the atmosphere here. By contrast, the interstellar version of this line is far from normal — it is observed without exception as a powerful maser whose pumping mechanism is not yet understood so that little information about the

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question of whether molecular clouds collapse, and at what rate, a question of direct consequence to the puzzle of how stars form from such clouds. Late last year Philips and his colleagues made perhaps the most important discovery yet from the KAO, the fine-structure (3P_1 - 3P_0) ground-state transition of neutral atomic carbon at 492 GHz (BAAS 11, 686; 1979). The line is apparently observable everywhere that CO is seen. Not only is carbon chemistry predominant in interstellar molecular clouds, but models predict extremely abrupt transitions in the ionized, neutral, and molecular forms of carbon as a function of optical depth of the cloud. Such predictions can now be checked. Neutral atomic carbon is also expected to be one of the major coolants of clouds by emission in its two fine structure transitions. A much better determination of heating-cooling mechanisms in these clouds is now possible. It is quite probable

that the new carbon line will take its place along with the atomic hydrogen line at 21 cm and the CO line at 2.6 mm as one of the three most important interstellar lines in the radio-infrared spectrum.

In the short time that observations of the interstellar medium from above the troposphere have been possible, the results have been quite remarkable. More of the same can be expected. Yet to come are searches for the hydrides of many elements, the other fine structure transition of carbon (at 812 GHz), important molecules such as HD and O_2 , and vibrationally excited molecular species of all kinds including several (such as acetylene and carbon dioxide) that because of their symmetry do not radiate at microwave frequencies. The KAO has been the vital first step in opening one of the last, and potentially richest, regions of the astronomical spectrum — that of the sub-millimetre and far infrared. □

Spinning yarns about plant cells

from Clive Lloyd

WHERE and how is cellulose made? With rare exception it doesn't seem to be made inside plant cells, neither does it appear to be spun-out from precursors away from the cell membrane. In fact, it has long been held that cellulose is synthesized at the plasma membrane but although convincing images exist of the sites of assembly in algae there is still little agreement about the nature of the synthesizing machinery in higher plants. Membrane fractions will add radioactive glucose (from nucleotide-glucose) to primer whereas unbroken protoplasts will not and so it is thought that the activated glucose must be donated to membrane-located cellulose synthetases at their cytoplasmic face. Splitting the lipid layer open by freeze-etch/freezefracture techniques should reveal any large transmembranous assemblies but it is precisely this area of producing artefact-free images of putative "assemblyases" which is fraught with problems and has prolonged the debate about what happens in higher plants.

It was in 1963 that Preston suggested the existence of enzyme particle complexes in the plasma membrane responsible for the synthesis of cellulose microfibrils and they were soon found by the new technique of freeze-etch/freezefracture (see Preston *The Physical Biology of Plant Cell Walls*, Chapman & Hall, 1974). With the algae *Oocystis* (Brown & Montezinos *Proc. natn. Acad. Sci. U.S.A.* 73, 143; 1976) and *Glaucozystis* (Willison & Brown *J. Cell Biol.* 78, 103; 1978) and the bacterium *Acetobacter xylinum* (Brown, Willison & Richardson *Proc. natn. Acad. Sci. U.S.A.* 73, 4565; 1976), intramembranous com-

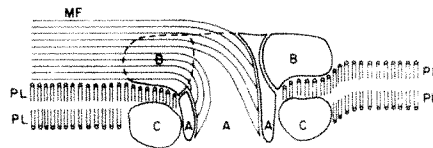


Fig. 1 A model demonstrating the proposed relationship of terminal complexes and rosettes in association with the plasma membrane. The membrane, microfibrils, and microfibril-associated structures are drawn approximately to equivalent scale. The dimensions of the rosettes (C), the terminal complex (A and B) and the microfibril (MF) are derived from freeze-fracture images. (from Mueller & Brown, *op. cit.*)

plexes revealed by this technique pack together in lines and are associated with cellulose microfibrils on the external face. Linear (as well as hexagonal) arrays of particles are also seen in fractured membranes of regenerating protoplasts from the higher plant *Skimmia japonica* (Robenek & Peveling *Planta* 136, 135; 1977) when glycerol is used. However, Davey and Mathias (*Protoplasma* 100, 85; 1979) have shown that the formation of both hexagonal and linear arrays of membrane particles can be attributed to the use of glycerol — a cryoprotectant known to induce similar artefacts in animal cells. So, do intramembranous assemblies participate in processing the higher plant cell wall or do they not? They do according to work from R.M. Brown's laboratory. In 1976, Mueller, Scott and Brown (*Science* 194, 949) presented pictures of the extracellular face of fractured membranes from untreated corn roots, showing globular complexes attached to the ends of nascent cellulose microfibrils. Willison and

Grout (*Planta* 140, 57; 1978) also avoided chemical fixation or glycerol treatment and showed similar globules attached to the ends of microfibrils in radish roots. Tomato and tobacco protoplasts were also studied but even though they were presumably regenerating a wall, no impressions of wall microfibrils were made into the membrane, thus preventing any correlation between cellulose microfibrils and the intramembranous particles. Without their walls, protoplasts are fragile and are prevented from swelling and bursting by regulating the osmotic concentration of the suspension medium with sugars. It seems likely, then, that in contrast to whole tissue, a wall in the process of being regenerated by a protoplast may be physically incapable of imprinting its image through to the membrane's fracture-plane where the particles are seen. For several reasons, therefore, it would seem that protoplasts may not be ideal for studying wall synthesis and now Wilkinson and Northcote (*J. Cell Science*, in the press) reinforce the message by reporting that even plasmolysis (a routine preliminary to preparing protoplasts from suspension cells) induces proteins to crystallize in the plane of the membrane as the area of surrounding lipid bilayer is reduced during retraction of the protoplast from the cell wall. It would seem that particle arrays produced by any treatment (not just glycerination) which causes plasmolysis could be artefactual.

This readiness of intramembranous particles to pack together under varied experimental conditions tends to sap confidence in the physiological relevance of ordered particle arrays to cellulose biosynthesis. It is satisfying therefore to find further good evidence in higher plant cells which favours the idea that specialized particle rosettes, quite unlike the more dubious hexagonal arrays, are associated with cellulose synthesis. Mueller and Brown (*J. Cell Biol.* 84, 315; 1980) have now confirmed the presence of such assemblies in untreated corn, pine and mung bean seedlings by freezing the material directly in solid nitrogen. They find that terminal complexes are associated with impressions made by cellulose microfibrils into the external leaflet of the lipid bilayer. It used to be said that cellulose formed a continuous meshwork without visible free ends but in one case a microfibril has been pulled through this leaflet but is still seen to terminate in the central subunit of such a complex. On the complementary protoplasmic side of the fracture, rosettes of membrane particles are commonly found. Both the rosettes and the terminal complexes are associated with the ends of microfibril impressions and it is envisaged that these could be complementary to one another such that the central subunit (which terminates the cellulose microfibril) normally sits within the rosette (Fig. 1). Once again, these investigators emphasize the importance of cellular

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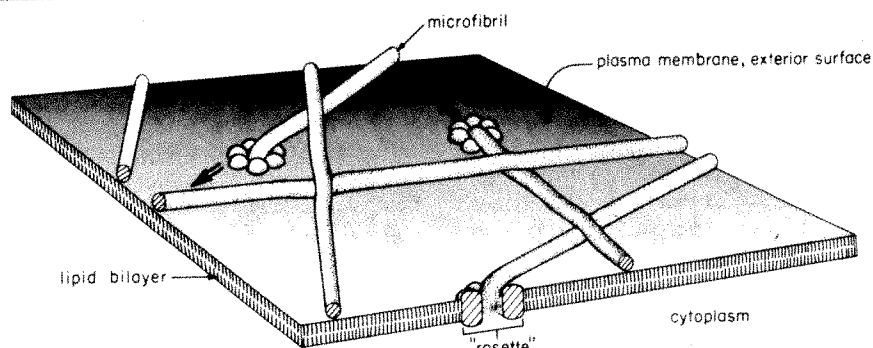


Fig 2 Model of microfibril deposition during primary wall formation in *Micrasterias*. Above: side view. Below: surface view. Single rosettes apparently give rise to randomly oriented microfibrils. (from Giddings *et al. op. cit.*)

turgor for visualizing the terminal complexes. A brief (1 min) exposure to glycerol before freezing causes the cells to lose turgor whereupon microfibril impressions are no longer observed. An accompanying paper by Giddings, Brower and Staehelin (*J. Cell Biol.* **84**, 327; 1980) proposes a similar view of cellulose biosynthesis drawn from work on the green alga *Micrasterias denticulata*. Here, the hexagonal rosette, together with the central microfibril-terminating subunit, are pictured as a transmembrane complex responsible for the elaboration of the basic single 5 nm cellulose microfibril (Fig. 2). Rows of such complexes are believed to form sets of microfibrils which aggregate laterally to form the larger fibrils composing the secondary wall. This view of wall formation in *Micrasterias* supports that of Kiermayer and Dobberstein (Protoplasma,

77, 437; 1979) who also indicated that these specialized areas of membrane are derived by the insertion of Golgi-derived 'flat vesicles' into the plasma membrane.

These investigators are properly cautious in designating these particle complexes as the cellulose synthesizing machinery. However, if correct, the next major question for plant morphogenesis is what determines the orientation of cellulose deposition and hence plant cell shape. Perhaps the linearity of crystalline cellulose fibrils is sufficient to steer through the membrane the ordered groups of particle complexes which extrude them. On the other hand, there are grounds to believe (in higher plants) that cytoplasmic microtubules, which may be bridged to the plasma membrane, influence the orientation of cellulose deposition — but that is another story. □

To understand this object, Cash *et al.* have considered its possible relation to other interesting objects in the same region of the galactic plane. A ring of optical filaments emitting $H\alpha$ coincides with the boundary of the X-ray ring. The HEAO-1 observers suggest that the same interstellar shock which has heated the interstellar gas to produce the X-rays may be responsible for exciting these optical filaments. A large expanding loop of neutral hydrogen, one of several reported by Carl Heiles on the basis of 21 cm observations (*Astrophys. J.* **229**, 533; 1979), lies in the same direction. The most important object in the region is the group of young stars known as the Cygnus OB2 association, which lies within the boundaries of the X-ray source.

This assembly of hot and luminous stars might provide the energy for the X-ray superbubble, either through a slow sustained energy input due to violent stellar winds or by impulsive energy input from supernova explosions of the most massive stars in the association. Cash *et al.* show that a single supernova explosion would require 10^{54} erg to create the X-ray source, an energy 10^3 times larger than any observed supernova. The energy demands need not be so severe if the supernovae are frequent enough. In that case, each successive event will take place in the low density cavity blown in the interstellar gas by its predecessor. In this way, a bubble of the immense size observed with HEAO-1 could be created by several successive supernova explosions in the OB association.

The Cygnus superbubble is not the only large region of the interstellar gas which has been observed to be filled with a hot low-density medium. Reynolds and Ogden (*Astrophys. J.* **229**, 942; 1979) have examined the energetics and kinematics of a 280 parsec diameter region which is connected to the I Orion OB association that excites the Orion Nebula. Just as in the Cygnus case, faint $H\alpha$ filaments and 21 cm features outline a large soft X-ray source. Because the Orion bubble is only 460 parsec away, this object covers a full 40° on the sky. The measurements of Reynolds and Ogden show that the optical filaments are expanding at about 20 km s^{-1} . The picture in Orion, as in Cygnus, seems to be that several stars from the OB association have already lived out their lives and detonated as supernovae, carving out a large, hot bubble in the interstellar gas from their overlapping shock waves.

The idea that a substantial fraction of the interstellar medium might be maintained at a temperature of 10^6 K and a density near 10^{-2} cm^{-3} by means of overlapping supernova remnants is not new. In 1974, Cox and Smith (*Astrophys. J. Lett.* **189**, 105; 1974) pointed out that supernova explosions are frequent enough that their remnants might often connect, and more recently McKee and Ostriker (*Astrophys. J.* **218**, 148; 1977) have developed a comprehensive model for the interstellar

Interstellar violence observed

from Robert Kirshner

SUPERSONIC shocks created by stellar winds or supernova explosions are capable of heating large volumes of the interstellar gas in our Galaxy to temperatures approaching those of the solar corona. Thermal emission from this torrid interstellar medium emerges as soft X rays in the vicinity of 1 keV. The A-2 experiment on the HEAO-1 satellite spent the time from its launch in August 1977 until its demise in January 1979 in a patient effort to construct an all-sky map in soft X rays. The virtue of this approach is that it can reveal new sources in unexpected places and that it can detect very large and nearby objects that are too diffuse for imaging detectors. This scheme has paid off handsomely in the discovery of a very large (13° diameter) soft X-ray source in the constellation Cygnus which may be the fossil remains of a series of violent explosions in the interstellar gas.

As reported at a recent American Astronomical Society Meeting (San Francisco, 16 January, 1980) Webster Cash

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of the University of Colorado and his collaborators from Berkeley, Caltech and the Jet Propulsion Laboratory have examined a portion of the soft X-ray survey. From their map of the Cygnus region, they find several known supernova remnants together with an immense horseshoe of emission they term an X-ray superbubble. Their data show that the emission comes from gas near 2 million K, and that there is substantial cold gas between us and the emission source which absorbs many of the X rays. From the absorption data, Cash *et al.* estimate that the distance to the giant loop of X-ray emission is about 2 kiloparsecs. Although the distance is uncertain, it implies that the diameter of the X-ray source is an astonishing 450 parsecs, 10 times larger than an ordinary supernova remnant. From the distance and the observed X-ray flux, they use the estimated temperature to derive an electron density of about 0.02 cm^{-3} , and a total thermal energy content of about $6 \times 10^{51} \text{ erg}$. This is about an order of magnitude larger than the total energy output of an ordinary supernova.

medium which predicts that coronal gas, in rough pressure equilibrium with cooler phases of interstellar gas, may fill a substantial fraction of the galactic disk. The HEAO-1 survey, and particularly the new Cygnus superbubble, provide direct evidence that the mechanisms envisioned by these theorists actually do take place. Both the Cygnus object and the Orion bubble emphasize that OB associations, where large collections of massive stars can be found, may play a prominent part in creating the overlapping supernova remnants that can fill large portions of the galaxy with coronal gas. □

Topical fusion

from M. Keith Matzen

EDWARD Teller (Lawrence Livermore Laboratory) opened a recent meeting on inertial confinement fusion (ICF)* by stressing the importance of research over premature emphasis on development. We were reminded that an ICF reactor is technologically more complex than a fission reactor, that it took 20 years to develop an economically feasible fission power plant after the demonstration of the first fission reactor, and that the first demonstration of an ICF reactor is many years away. Teller believes that there is a "small chance" that a magnetic fusion reactor could be built in this century, but that the chances of an ICF reactor in the same time are "extremely remote". On the other hand he believes that a magnetic fusion-fission hybrid could be built in about 10 years and should be pursued as an energy-producing alternative.

According to Lawrence Killion (Department of Energy, Office of Inertial Fusion) the emphasis in the ICF programme during the next decade will be to define an appropriate driver. He stated that lasers are no longer considered necessarily to be the dominant long-term driver because of their high cost and the uncertainties in the laser-target coupling. The goals for the programme in the 1980s must include the demonstration of a high-gain driver-target system, the determination of the size of a reactor driver, the understanding of driver-target coupling, the achievement of breakthroughs in driver technology, and the achievement of breakthroughs in target fabrication technology.

John Nuckolls (LL) provided further requirements and perspectives for future ICF research. The basic requirement of an ICF reactor system is an energy gain of greater than 100 with a driver energy of less than 10 MJ (10^7 Joules). Computer calculations now predict that an energy gain of 100 can be obtained with driver

energies of 2 MJ (\pm a factor of 3). He stated this energy requirement had not changed in several years, in contrast to the inflation of the driver energy required for breakeven (gain 1) targets. The increase in the break-even driver energy from 1 kJ (see *Nature* 239, 139; 1972) to the present estimate of 300 kJ (\pm a factor of 3) is due to the inability to approach the theoretical limits of the early calculations, both in laser technology and in laser-target coupling physics.

The importance of understanding the laser-target coupling physics can be illustrated by a number of key theoretical issues: fast electron transport, competition between Brillouin scattering and inverse bremsstrahlung absorption, production of fast electrons, and beam filamentation. He suggested that future experimental work should emphasize studies with longer pulse lengths and shorter wavelengths, measurements of the underdense plasma, studies of the fast electron production and transport, and studies of bandwidth effects. Although ions (both heavy and light) must solve problems of achieving sufficient power density on target, their target-coupling problems seem minimal. There is a rigorous upper limit for the ion range in a target and there are apparently no problems with fuel preheat from the electron and nuclear reactions. The problem of transporting ion beams to the target is difficult but seems to be soluble. In summary, Nuckolls gave the following estimates for the parameters of a driver for an ICF reactor: 3 MJ energy, 150 TW power, 5-10% efficient, beam focusable to less than 5 mm, 5 Hz repetition rate, cost less than 10^9 dollars, and operate for 10^{10} shots. The candidate reactor drivers are short wavelength lasers (KrF for example), heavy ion beams (HIB), and light ion beams (LIB). The drivers that may show target ignition (DT gain ~ 5 , target gain ~ 0.1) in the mid 1980s are Nova (at Lawrence Livermore Labs) and PBFA (at Sandia National Labs).

The recent emphasis in experimental programmes has been in the areas of intermediate density targets (10 times liquid DT density), techniques for measuring these compressed densities, X-ray backlighting techniques, ablative acceleration of thin foils, characterizing the back-reflected laser light, characterizing the thermal conductivity and fast-electron production by using multiple-layer targets, measuring the driver-target coupling efficiency, and experiments to examine the stability of implosion systems. With the trend towards shorter wavelength laser-drivers, several laboratories have begun experiments with doubled, tripled, and quadrupled glass laser light (wavelengths of 0.53, 0.35, and 0.26 μm , respectively). Although most experiments are still in their initial stages, the early results generally show better coupling to the target, decreased fast electron production, and better thermal conduction

as the laser wavelength decreases. However, Fred Mayer (KMS Fusion) presented measurements in which the same absorption was observed for 0.53 μm and 1.06 μm irradiation of spherical targets with 60 to 100 ps pulses. These results are in contrast to the strong wavelength dependence observed in 0.26 μm , 0.53 μm , and 1.06 μm irradiation of planar targets (Fabre *et al.*, Ecole Polytechnique Palaiseau, France). The near future will see a great deal of effort devoted to short-wavelength ($\leq 0.53 \mu\text{m}$) irradiation experiments.

ICF reactor designs are still in the conceptual stages. Protection of the reactor walls from the radiation environment is a major consideration. Several reactor designs have been considered, although flowing-liquid-metal-walled reactors have received most attention and represent the only design in which protection is provided against all the fusion reaction products. Jerry Kulcinski (University of Wisconsin) reported that work is needed in the areas of fusion reactor economics, safety and environmental impact, as well as in recovery of pellet debris, protection of final focusing elements, small LIB reactors, minimum sized HIB reactors, and pulsed neutron damage. Both Kulcinski and Noel Amherd (EPRI) stated that LIB technology appears to offer the only approach to small ICF reactors at this time. John Caird (Bechtel National, Inc.) has projected the capital cost of a HIB power plant to be from 4.2 to 6.6 billion dollars for plants in the range from 1.125 to 2.25×10^9 Watts-electrical.

With the trend towards larger, more complicated high-gain targets, the technology of target fabrication continues to be challenged. High-gain targets, in general, consist of multiple shells and larger aspect ratios (ratio of spherical shell radius to shell thickness). Thus the questions of target-shell finish and stability of implosion become more important. The technology for building these multishell targets is not yet available. At this meeting, the emphasis was on target-shell and fuel-fill characterization techniques, pellet fabrication methods and mass production of pellets. Novel approaches to target fabrication include using a low-gravity environment to form large-diameter spherical bubbles, laser drilling of 2 to 4 μm holes in microballoons, followed by gas filling and hole plugging, and structural modification of polymers to reduce crystallinity and improve the surface finish. Nondestructive fuel fill characterizations is another area of active research.

Progress on the construction of new, larger drivers continues. Nova, the 100 kJ, 1-3 ns glass facility at Lawrence Livermore Laboratory should be available for target

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*The topical meeting on Inertial Confinement Fusion (ICF) was held in conjunction with the Conference on Laser and Electro-Optical Systems (CLEOS) in San Diego, California, February 26-28, 1980.

experiments in 1984. An interesting sidenote is that Shiva was operational 1 month after the earthquake in Livermore and suffered only minor damage to the optical system. At Los Alamos, the current scenario for the Antares CO₂ laser facility envisages the construction of two of the previously proposed six beam lines so that 40 kJ of CO₂ laser energy can be provided for target experiments in 1983. Recent improvements in the triple-pass amplifiers of the Helios system at Los Alamos will increase their energy on target from 5 to 10 kJ by early summer. The light-ion-beam facility at Sandia Laboratories (PBFA-1) is scheduled for completion this year and will provide 1 MJ and 30 TW. The upgrade of this facility, PBFA-II, will deliver 3.5 MJ at 100 TW and will be available for experiments in 1986. A 10-kJ heavy-ion-beam target physics facility has been proposed for construction and operation by 1984. As mentioned previously the programme for new laser development is emphasizing short wavelength lasers. The present candidates are the rare gas halide lasers KrF, XeCl, and XeF. All these require a pulse compression technique to obtain pulses in the neighbourhood of 20 ns. Papers at this meeting showed the feasibility of both Raman compression and pulse stacking (angular multiplexing) to achieve shorter pulses. A major drawback of these short-wavelength systems is the low damage threshold of the optical components and the resulting high cost of the optical system. This problem has not been adequately addressed. Since the tradeoffs between these systems will depend very strongly on the results of future laser-target coupling experiments, the option to use longer-wavelength lasers has not been eliminated. □

Thunderstorms and substorms: any connection?

from D. J. Southwood

It is a familiar fact that substantial electric fields are produced in the atmosphere during a thunderstorm. As well as a steady (d.c.) field being present, thunderstorm lightning flashes are a source of transient a.c. electromagnetic signals over a wide frequency range. A less familiar electromagnetic storm phenomenon is the magnetospheric substorm, a disturbance of the ionosphere and magnetosphere in the tenuous outer ionized layers of the Earth's atmosphere. The substorm's most dramatic manifestation is in intense

auroral displays. The aurora results from the strong electric coupling between the magnetosphere and the ionosphere below, a process which can require strong currents to flow between them. The aurora occurs when the currents are carried by energetic electron beams which give rise to light as they strike the denser lower ionosphere.

The ionosphere and magnetosphere are very good electrical conductors while the troposphere, the lowest layer of the atmosphere and the one in which thunderstorms occur, is insulating. There have been few attempts to consider whether there could be any significant electrical coupling between the troposphere and the outermost regions except by those workers (Markson *Nature* 273, 103; 1978; Herman & Goldberg *J. Atmos. Terr. Phys.* 40, 121; 1978) looking at "sun-weather relationships". The search is for causal links between activity in the tenuous outer layers of the atmosphere and the much denser bottom 5-10 km where the weather occurs. Any such connection must be subtle. The energy associated with tropospheric motions far exceeds that associated with any magnetospheric effect. Electric coupling (Markson *op. cit.*) is one possible connection.

Data from a recently reported balloon flight (Bering *et al. J. geophys. Res.* 85, 55; 1980) in northern Canada that took place during the simultaneous occurrence of a thunderstorm and a magnetospheric substorm has permitted a direct look at some interrelations between these apparently disparate disturbances in very different regions. The balloon was some 10-20 km above the top of the thunderstorm activity (at ~ 10 km) but some 60 to 70 km below the bottom of the E region ionosphere. The thunderstorm was localised and centred some 30-40 km north-west of the balloon launch site in Roberval, Quebec. In contrast the substorm was a worldwide event. Magnetograms from the USSR, Alaska, Canada and Greenland showed that magnetic perturbations due to the magnetospheric substorm were present all around the auroral zone. The balloon was instrumented to measure vertical and horizontal electric fields, electrical conductivity and X-ray emissions. The latter provide information on energetic electrons precipitated from the magnetosphere but which are stopped at much higher altitudes than the balloon's flight height. On the ground VLF (very low frequency) radio receivers, goniometers and other equipment for support measurements were deployed.

The d.c. electric field at the balloon was dominated by the presence of the thunderstorm. There are uncertainties in extrapolating the observed field upwards to the ionosphere. On this flight a reasonable estimate suggests the thunderstorm charge distribution should produce a field of 10^{-7} V m⁻¹ in the ionosphere. In contrast the detection of

echoes by the Cornell University auroral radar which was operating during the flight shows the actual ionospheric field must have exceeded 10^{-2} V m⁻¹. It seems safe to say the thunderstorm electric field had no effect on the magnetosphere and ionosphere.

Thunderstorms are a strong source of a.c. signals in the atmosphere and these can penetrate upwards far more effectively. Lightning flashes associated with the storm generate ULF (ultra low frequency) and VLF electromagnetic noise. In the 1-30 kHz VLF band lightning-flashes have long been known as a source of whistler mode plasma waves in the magnetosphere. A complicated interaction between magnetosphere and atmosphere can then be envisaged because lightning-launched whistler mode signals can interact with the magnetospheric energetic electron population. In particular, waves can scatter electrons onto orbits which bring them down into the atmosphere or lower ionosphere along the Earth's magnetic field. Here they experience collisions and in particular generate secondary X rays. The X-ray emissions seen on the balloon flight were of the microburst type, short bursts (< 1 s) which characteristically occur during substorms. A key question thus was, were any microbursts caused by lightning-induced VLF? The authors argue there were. Their test is to look at how many microbursts occur within 1.5 s of a lightning flash. 62 out of 95 nearby flashes appeared to have closely correlated bursts of electron precipitation following them. The authors estimate a 1% probability of this being chance.

The flashes also produce substantial power in the much lower ULF band (< 1 Hz). They conclude this could have a substantial effect in the ionosphere and suggest the thunderstorm-induced ionospheric ULF field could considerably exceed the substorm-induced ULF fields in the ionosphere. Whether this is of significance in magnetospheric terms is another matter. No measurements were made of magnetospheric parameters that could have been directly affected (such as ion precipitation).

The authors' most surprising measurement is that of the local electrical conductivity. It is about half its fair weather value. This certainly contradicts electrical coupling mechanisms between atmosphere and magnetosphere which invoke a substorm-induced conductivity enhancement as a trigger in a coupling mechanism.

All in all the report cuts down our ability to speculate on electric coupling of magnetosphere and atmosphere. D.C. coupling seems unimportant. The conductivity measurement is a puzzle. It does seem ULF and VLF phenomena can couple but such coupling should not be of dynamic significance in either region. The hard fact is we seem no nearer a substantive connection. □

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Distributed feedback lasers

from R.G. Harrison

SINCE the gain and so the output power of most gas lasers increases as the inner diameter of the laser tube decreases, considerable attention has been given to the design of laser systems with very narrow bores. For these lasers the electromagnetic resonator modes are altered in a fundamental way from those obtained from conventional open resonator cavities formed by mirrors; the radiation field distribution being no longer determined by the mirrors but by the modes of the hollow waveguide. (Marcatili & Schmetzer, *Bell Syst. Tech. J.*, **43**, 1783; 1964). Laser oscillation is normally sustained in such systems by feedback to the tube from external mirrors. However, an alternative method recently demonstrated is that of distributed feedback (DFB) by which a forward propagating mode is coupled to its corresponding backward mode through scattering from periodic diameter or width changes in the tube. The distinctive feature of this coupling is that the scattered wave excited at a certain position in the waveguide is added in phase to the already existing wave, efficient coupling occurring only when the frequency of the ripple structure in the tube is nearly equal to twice the propagation constant of the lasing mode. Since it is not necessary that this structure be sinusoidal a convenient alternative can be provided by rectangular grooves, coupling then being provided by the fundamental frequency of the periodic rectangular ripple.

For distributed feedback systems the threshold for oscillation increases as the order of resonance of the resonant mode increases. Thus they offer the advantage of operation on the lowest order waveguide mode at a single frequency determined by the resonant mode q ; discrimination from other modes being controlled by choosing the gain of the laser such that all higher order modes than the lowest one suffer overall loss. This together with low laser thresholds and the requirement of no external optics makes the technique potentially attractive. Furthermore, the method should not be constrained by the material or cross-sectional geometry of the light tube, and as such it may be applied to many gas laser systems. These include optically pumped lasers for the near to far infrared using either dielectric or metal tubing and also transverse electrically excited high pressure systems and Stark tuned optically pumped lasers, both comprising parallel flat electrodes separated by dielectric spacers to form a rectangular section, metal-dielectric, waveguide.

Distributed feedback laser action was first reported some years back by Kogelnik

and Shank (*Appl. Phys. Letts.*, **8**, 152; 1971) for a dye system. However it was only recently that such action was achieved in a gas system (Affolter & Kneubuhl, *Phys. Lett.*, **74A**, 407; 1979) even though the theoretical foundations for such operations had been well established (Marcuse, *IEEE J. Quantum Electr.*, **QE-10**, 413, 1974; Kneubuhl, *J. Opt. Soc. Am.*, **11**, 1; 1976). Part of the reason lies in matching the laser emission with the periodic structure of the DFB waveguide. For both dye and solid state lasers there is no problem since the emission linewidth is relatively large. However, for both optically pumped and electrically excited gas lasers, usually operated at low pressures, this is not so and matching is correspondingly critical. In the preliminary work of Affolter and Kneubuhl this difficulty has been overcome by temperature tuning the periodic structure of a CH_3F optically pumped waveguide system over a temperature range between 30°C and 90°C , the temperature variation of 10°C corresponding to a relative change of $\sim 10^{-4}$ of the waveguide period. The bottom of the waveguide was periodically corrugated with rectangular grooves of depth $124\text{ }\mu\text{m}$ and period $248\text{ }\mu\text{m}$,

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corresponding to a quarter and a half of the wavelength of the CH_3F emission and feedback was optimized by varying the height of the waveguide.

Evidence of DFB action was demonstrated in the resonant or mode features of the output energy of the CH_3F $496\text{ }\mu\text{m}$ emission as a function of the waveguide temperature. Single mode emission was obtained with optimum output at the centre of the temperature range. For higher pump energies oscillation of two modes was observed, peaked at temperatures separated by around 45°C , corresponding to a mode separation of 450 MHz and in agreement with DFB theory. The relatively broad temperature bandwidth of around 20°C (FWHM) over which oscillation occurs on a single mode, together with the readily accessible temperature range used in the tuning, are both particularly attractive features of the method with regard to control and operation of the system.

Whether the distributed feedback technique will eventually replace conventional cavity optics in some laser systems remains to be seen. Nevertheless these new results are promising and as such may provide the key to an accelerated effort into the research and development of such systems in the near future. □



100 years ago

In *Nature*, vol. xxi. p. III, we described the ingenious planetarium recently invented by Signor Perini, and which has cost him seven years' constant labour. To-day we are able to present an illustration of this invention, which may give those of our readers who have not seen the original, some idea of its construction. The visitors are supposed to be standing underneath the dome, from which are suspended the sun and planets.



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REVIEW ARTICLES

Selfish genes, the phenotype paradigm and genome evolution

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Natural selection operating within genomes will inevitably result in the appearance of DNAs with no phenotypic expression whose only 'function' is survival within genomes. Prokaryotic transposable elements and eukaryotic middle-repetitive sequences can be seen as such DNAs, and thus no phenotypic or evolutionary function need be assigned to them.

THE assertion that organisms are simply DNA's way of producing more DNA has been made so often that it is hard to remember who made it first. Certainly, Dawkins has provided the most forceful and uncompromising recent statement of this position, as well as of the position that it is the gene, and not the individual or the population, upon which natural selection acts¹. Although we may thus view genes and DNA as essentially 'selfish', most of us are, nevertheless, wedded to what we will call here the 'phenotype paradigm'—the notion that the major and perhaps only way in which a gene can ensure its own perpetuation is by ensuring the perpetuation of the organism it inhabits. Even genes such as the segregation-distorter locus of *Drosophila*², 'hitch-hiking' mutator genes in *Escherichia coli*^{3,4} and genes for parthenogenetic reproduction in many species⁴—which are so 'selfish' as to promote their own spread through a population at the ultimate expense of the evolutionary fitness of that population—are seen to operate through phenotype.

The phenotype paradigm underlies attempts to explain genome structure. There is a hierarchy of types of explanations we use in efforts to rationalize, in neo-darwinian terms, DNA sequences which do not code for protein. Untranslated messenger RNA sequences which precede, follow or interrupt protein-coding sequences are often assigned a phenotypic role in regulating messenger RNA maturation, transport or translation⁵⁻⁷. Portions of transcripts discarded in processing are considered to be required for processing⁸. Non-transcribed DNA, and in particular repetitive sequences, are thought of as regulatory or somehow essential to chromosome structure or pairing⁹⁻¹¹. When all attempts to assign to a given sequence or class of DNA functions of immediate phenotypic benefit to the organism fail, we resort to evolutionary explanations. The DNA is there because it facilitates genetic rearrangements which increase evolutionary versatility (and hence long-term phenotypic benefit)¹²⁻¹⁷, or because it is a repository from which new functional sequences can be recruited^{18,19} or, at worst, because it is the yet-to-be eliminated by-product of past chromosomal rearrangements of evolutionary significance^{9,19}.

Such interpretations of DNA structure are very often demonstrably correct; molecular biology would not otherwise be so fruitful. However, the phenotype paradigm is almost tautological; natural selection operates on DNA through organismal phenotype, so DNA structure must be of immediate or long-term (evolutionary) phenotypic benefit, even when we

cannot show how. As Gould and Lewontin note, 'the rejection of one adaptive story usually leads to its replacement by another, rather than to a suspicion that a different kind of explanation might be required. Since the range of adaptive stories is as wide as our minds are fertile, new stories can always be postulated' (ref. 20).

Non-phenotypic selection

What we propose here is that there are classes of DNA for which a 'different kind of explanation' may well be required. Natural selection does not operate on DNA only through organismal phenotype. Cells themselves are environments in which DNA sequences can replicate, mutate and so evolve²¹. Although DNA sequences which contribute to organismal phenotypic fitness or evolutionary adaptability indirectly increase their own chances of preservation, and may be maintained by classical phenotypic selection, the only selection pressure which DNAs experience directly is the pressure to survive within cells. If there are ways in which mutation can increase the probability of survival within cells without effect on organismal phenotype, then sequences whose only 'function' is self-preservation will inevitably arise and be maintained by what we call 'non-phenotypic selection'. Furthermore, if it can be shown that a given gene (region of DNA) or class of genes (regions) has evolved a strategy which increases its probability of survival within cells, then no additional (phenotypic) explanation for its origin or continued existence is required.

This proposal is not altogether new; Dawkins¹, Crick⁶ and Bodmer²² have briefly alluded to it.

However, there has been no systematic attempt to describe elements of prokaryotic and eukaryotic genomes as products of non-phenotypic selection whose primary and often only function is self-preservation.

Transposable elements in prokaryotes as selfish DNA

Insertion sequences and transposons can in general be inserted into a large number of chromosomal (or plasmid) sites, can be excised precisely or imprecisely and can engender deletions

or inversions in neighbouring chromosomal (or plasmid) DNAs¹²⁻¹⁶. These behaviours and, at least in some cases, the genetic information for the enzymatic machinery involved, must be inherent in the primary sequences of the transposable elements themselves, which are usually tightly conserved^{12-16,23}. Most speculations on the function of transposable elements concentrate on the role these may have, through chromosomal rearrangements and the modular assembly of different functional units, in promoting the evolution of plasmid and bacterial chromosomes, and thus in promoting long-term phenotypic fitness¹²⁻¹⁶. Most assume that it is for just such functions that natural selection has fashioned these unusual nucleic acid sequences.

Although transposable elements may well be beneficially involved in prokaryotic evolution, there are two reasons to doubt that they arose or are maintained by selection pressures for such evolutionary functions.

First, DNAs without immediate phenotypic benefit are of no immediate selective advantage to their possessor. Excess DNA should represent an energetic burden^{24,25}, and some of the activities of transposable elements are frankly destructive¹²⁻¹⁶. Evolution is not anticipatory; structures do not evolve because they might later prove useful. The selective advantage represented by evolutionary adaptability seems far too remote to ensure the maintenance, let alone to direct the formation, of the DNA sequences and/or enzymatic machinery involved. A formally identical theoretical difficulty plagues our understanding of the origin of sexual reproduction, even though this process may now clearly be evolutionarily advantageous^{1,4}.

Second, transposability itself ensures the survival of the transposed element, regardless of effects on organismal phenotype or evolutionary adaptability (unless these are sufficiently negative). Thus, no other explanation for the origin and maintenance of transposable elements is necessary. A single copy of a DNA sequence of no phenotypic benefit to the host risks deletion, but a sequence which spawns copies of itself elsewhere in the genome can only be eradicated by simultaneous multiple deletions. Simple translocation (removal from one site and insertion into another) does not provide such insurance against deletion. It is significant that recent models for transposition require retention of the parental sequence copy^{26,27}, and that bacterial insertion sequences are characteristically present in several copies per genome¹⁶. The assumption that transposable elements are maintained by selection acting on the cell does not require that they show these characteristics. The evolutionary behaviour of individual copies of transposable elements within the environment represented by a bacterial genome and its descendants can be understood in the same terms as organismal evolution. Replicate copies of a given element may diverge in sequence, but at least those features of sequence required for transposition will be maintained by (non-phenotypic) selection; copies which can no longer be translocated will eventually suffer elimination. Some divergent copies may be more readily transposed; these will increase in frequency at the expense of others. Transposable elements which depend on host functions run the risk that host mutants will no longer transpose them; it is significant that at least some transposition-specific functions are known to be coded for by the transposable elements themselves²⁶⁻²⁹. It is not to the advantage of a transposable element coding for such functions to promote the transposition of unrelated elements; the fact that given transposable elements generate flanking repeats^{16,30} of chromosomal DNAs of sizes characteristic to them (that is, 5, 9 or 11-12 base pairs) may indicate such a specificity in transposition mechanism. It is to the advantage of any transposable element to acquire genes which allow independent replication (to become a plasmid), promote host mating (to become a self-transmissible plasmid) or promote non-conjugational transmission (to become a phage like Mu).

It is certainly not novel to suggest that prokaryotic transposable elements behave in these ways, or to suggest that more frankly autonomous entities like phages have arisen from

them^{12-16,31}. However, we think it has not been sufficiently emphasized that non-phenotypic selection may inevitably give rise to transposable elements and that no phenotypic rationale for their origin and continued existence is thus required.

Transposable elements in eukaryotes

There has long been genetic evidence for the existence in eukaryotic genomes of transposable elements affecting phenotype³². These have been assigned roles in the regulation of eukaryotic gene expression and in evolution, but would have escaped genetic detection had they not had phenotypic effect. More recent evidence for transposable elements whose effects are not readily identified genetically has come fortuitously from studies of cloned eukaryotic DNAs. For instance, the Ty-1 element of yeast (which has no known phenotypic function) is flanked by direct repeats (like some prokaryotic transposons) and is transposable³³. It is present in some 35 dispersed copies and comprises some 2% of the yeast genome (like a higher-eukaryotic middle-repetitive DNA). The directly repeated δ -sequence elements flanking it are found at still other sites (just as prokaryotic insertion sequences can be found flanking transposons or independently elsewhere in the genome). Cameron *et al.* suggest that 'Ty-1 may be a nonviral "parasitic" DNA' but then go on to suggest, we think unnecessarily, that transposition 'allows adaptation of a particular cell to a new environment' (ref. 33). The repetitive elements 412, copia and 297 of *Drosophila* are physically similar to Ty-1 (and to bacterial transposable elements) and are transposable³⁴⁻³⁷. Strobel *et al.* suggest 'it is possible that the sole function of these elements is to promote genetic variability, and that their gene products may only be necessary for the maintenance and mobility of the elements themselves, rather than for other cellular processes' (ref. 37). But if maintenance and mobility mechanisms exist, then no cellular function at all need be postulated.

A large fraction of many eukaryotic genomes consists of middle-repetitive DNAs, and the variety and patterns of their interspersions with unique sequence DNA make no particular phylogenetic or phenotypically functional sense. Britten, Davidson and collaborators have elaborated models which ascribe regulatory functions to middle-repetitive DNAs, and evolutionary advantage (in terms of adaptability) to the quantitative and qualitative changes in middle-repetitive DNA content observed even between closely related species^{17,38-40}. Middle-repetitive DNAs are more conserved in sequence during evolution than are unique-sequence DNAs not coding for protein, and Klein *et al.* suggest that 'restraint on repetitive sequence divergence, either within the repeat families of a given species, or over evolutionary time spanning the emergence of different species, could be due to [phenotypic] selective pressures which prevent free sequence change in a large fraction of the repeat family members. Or perhaps repetitive sequences diverge as rapidly as do other sequences, but the type sequence of the family is preserved by frequent remultiplication of the "correct" surviving sequences' (ref. 41). The evidence for a phenotypically functional role for middle-repetitive sequences remains dishearteningly weak⁴⁰⁻⁴³, and if the calculations of Kimura⁴⁴ and Salser and Isaacson⁴⁵ are correct, middle-repetitive DNAs together comprise too large a fraction of most eukaryotic genomes to be kept accurate by darwinian selection operating on organismal phenotype. The most plausible form of "remultiplication of the 'correct' surviving sequences" is transposition. If we assume middle-repetitive DNAs in general to be transposable elements or degenerate (and no longer transposable and ultimately to be eliminated) descendants of such elements, then the observed spectra of sequence divergence within families and changes in middle-repetitive DNA family sequence and abundance can all be explained as the result of non-phenotypic selection within genomes. No cellular function at all is required to explain either the behaviour or the persistence of middle-repetitive sequences as a class.

The rest of the eukaryotic genome

Middle-repetitive DNA can comprise more than 30% of the genome of a eukaryotic cell⁴⁶. Another 1–40% consists of simple reiterated sequences whose functions remain unclear¹⁰, and Smith has argued that 'a pattern of tandem repeats is the natural state of DNA whose sequence is not maintained by selection' (ref. 47). Even unique-sequence eukaryotic DNA consists in large part of elements which do not seem to be constrained by phenotypic selection pressures⁴⁵. Some authors have argued that the intervening sequences which interrupt many eukaryotic structural genes are insertion sequence-like elements^{6,48,49}. If they are, they are likely to be the degenerate and no-longer-transposable descendants of transposable sequences whose insertion was rendered non-lethal by pre-existing cellular RNA:RNA splicing mechanisms. Such elements, once inserted, are relatively immune to deletion (since only very precise deletion can be non-lethal), and need retain only those sequence components required for RNA splicing. The rest of the element is free to drift and one expects (and observes) that only the position and number of intervening sequences in a family of homologous genes remain constant during evolution. Although evolutionary and regulatory phenotypic functions have been ascribed to intervening sequences^{6,49–51}, it is unnecessary to postulate any cellular function at all if these elements are indeed degenerate transposable elements arising initially from non-phenotypic selection. Another explanation for the origin and continued existence of intervening sequences, which also does not require phenotypically or evolutionarily advantageous roles, has been suggested elsewhere^{50,51}.

Why do prokaryotes and eukaryotes differ?

It is generally believed that prokaryotic genomes consist almost entirely of unique-sequence DNA maintained by phenotypic selection, whereas the possession of 'excess' unique and repetitive DNA sequences whose presence is at least difficult to rationalize in phenotypic terms is characteristic of eukaryotes. However, it is more accurate to say that there is a continuum of excess DNA contents; at least 1% of the *E. coli* genome can be made up of copies of six identified insertion sequences alone¹⁶. Yeast, whose genome is no larger than that of some prokaryotes, has few repeated sequences other than those coding for stable RNAs, and *Aspergillus* may have none^{52,53}. There is in general (but with many exceptions) a positive correlation between excess DNA content, genome size and what we anthropocentrically perceive as 'evolutionary advancement'. Many interpret

this as the cause and/or consequence of the increasing phenotypic complexity which characterizes organismal evolution, and attribute to excess DNA a positive role in the evolutionary process^{17–19,40}. The interplay of phenotypic and non-phenotypic forces, and the importance of understanding both in attempts to restore the 'C-value paradox' are discussed more thoroughly by Orgel and Crick in the following article.⁵⁴

There is another, simpler and perhaps obvious explanation. Non-phenotypic selection produces excess DNA, and excess DNA logically must be an energetic burden; phenotypic selection should favour its elimination^{24,25}. The amount of excess (and hence total) DNA in an organism should be loosely determined by the relative intensities of the two opposing sorts of selection. The intensity of non-phenotypic pressure on DNA to survive even without function should be independent of organismal physiology. The intensity of phenotypic selection pressure to eliminate excess DNA is not, this being greatest in organisms for which DNA replication comprises the greatest fraction of total energy expenditure. Prokaryotes in general are smaller and replicate themselves and their DNA more often than eukaryotes (especially complex multicellular eukaryotes). Phenotypic selection pressure for small 'streamlined' prokaryotic genomes with little excess DNA may be very strong.

Necessary and unnecessary explanations

We do not deny that prokaryotic transposable elements or repetitive and unique-sequence DNAs not coding for protein in eukaryotes may have roles of immediate phenotypic benefit to the organism. Nor do we deny roles for these elements in the evolutionary process. We do question the almost automatic invocation of such roles for DNAs whose function is not obvious, when another and perhaps simpler explanation for their origin and maintenance is possible. It is inevitable that natural selection of the special sort we call non-phenotypic will favour the development within genomes of DNAs whose only 'function' is survival within genomes. When a given DNA, or class of DNAs, of unproven phenotypic function can be shown to have evolved a strategy (such as transposition) which ensures its genomic survival, then no other explanation for its existence is necessary. The search for other explanations may prove, if not intellectually sterile, ultimately futile.

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Selfish DNA: the ultimate parasite

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The DNA of higher organisms usually falls into two classes, one specific and the other comparatively nonspecific. It seems plausible that most of the latter originated by the spreading of sequences which had little or no effect on the phenotype. We examine this idea from the point of view of the natural selection of preferred replicators within the genome.

THE object of this short review is to make widely known the idea of selfish DNA. A piece of selfish DNA, in its purest form, has two distinct properties:

- (1) It arises when a DNA sequence spreads by forming additional copies of itself within the genome.
- (2) It makes no specific contribution to the phenotype.

This idea is not new. We have not attempted to trace it back to its roots. It is sketched briefly but clearly by Dawkins¹ in his book *The Selfish Gene* (page 47). The extended discussion (pages 39–45) after P. M. B. Walker's article² in the CIBA volume based on a Symposium on Human Genetics held in June 1978 shows that it was at that time already familiar to Bodmer, Fincham and one of us. That discussion referred specifically to repetitive DNA because that was the topic of Walker's article, but we shall use the term selfish DNA in a wider sense, so that it can refer not only to obviously repetitive DNA but also to certain other DNA sequences which appear to have little or no function, such as much of the DNA in the introns of genes and parts of the DNA sequences between genes. The catch-phrase 'selfish DNA' has already been mentioned briefly on two occasions^{3,4}. Doolittle and Sapienza⁵ (see the previous article) have independently arrived at similar ideas.

The amount of DNA

The large amounts of DNA in the cells of most higher organisms and, in particular, the exceptionally large amounts in certain animal and plant species—the so-called *C* value paradox—has been an unsolved puzzle for a considerable period (see reviews in refs 6–8). As is well known, this DNA consists in part of 'simple' sequences, an extreme example of which is the very large amounts of fairly pure poly d(AT) in certain crabs. Simple sequences, which are situated in chromosomes largely but not entirely in the heterochromatin, are usually not transcribed. Another class of repetitive sequences, the so-called 'intermediate repetitive', have much longer and less regular repeats. Such sequences are interspersed with 'unique' DNA at many places in the chromosome, the precise pattern of interspersions being to some extent different in different species. Leaving aside genes which code for structural RNA of one sort or another (such as transfer RNA and ribosomal RNA), which would be expected to occur in multiple copies (since, unlike protein, their final products are the result of only one stage of magnification, not two), the majority of genes coding for proteins appear to exist in 'single' copies, meaning here one or a few. A typical example would be the genes for α -globin, which occur in one to three copies and the human β -like globins, of which there are four main types, all related to each other but used for slightly different purposes. Notable exceptions are the proteins of the immune system, and probably those of the histocompatibility and related systems. Another exception is the genes for the five major types of histone which also occur in multiple copies. Even allowing for all such special case, the estimated number of genes in the human genome appears too few to account for the 3×10^9 base pairs found per haploid set of DNA, although it must be admitted that all such arguments are very far from conclusive.

Several authors^{8–13} have suggested that the DNA of higher organisms consists of a minority of sequences with highly specific

functions plus a majority with little or no specificity. Even some of the so-called single-copy DNA may have no specific function. A striking example comes from the study of two rather similar species of *Xenopus*. These can form viable hybrids, although these hybrids are usually sterile. However, detailed molecular hybridization studies show that there has been a large amount of DNA sequence divergence since the evolutionary separation of their forebears. These authors¹³ conclude 'only one interpretation seems reasonable, and that is that the specific sequence of much of the single-copy DNA is not functionally required during the life of the animal. This is not to say that this DNA is functionless, only that its specific sequence is not important'.

There is also evidence to suggest that the majority of DNA sequences in most higher organisms do not code for protein since they do not occur at all in messenger RNA (for reviews see refs 14, 15). Nor is it very plausible that all this extra DNA is needed for gene control, although some portion of it certainly must be.

We also have to account for the vast amount of DNA found in certain species, such as lilies and salamanders, which may amount to as much as 20 times that found in the human genome. It seems totally implausible that the number of radically different genes needed in a salamander is 20 times that in a man. Nor is there evidence to support the idea that salamander genes are mostly present in about 20 fairly similar copies. The conviction has been growing that much of this extra DNA is 'junk', in other words, that it has little specificity and conveys little or no selective advantage to the organism.

Another place where there appears to be more nucleic acid than one might expect is in the primary transcripts of the DNA of higher organisms which are found in the so-called heteronuclear RNA. It has been known for some time that this RNA is typically longer than the messenger RNA molecules found in the corresponding cytoplasm. Heteronuclear RNA contains these messenger RNA sequences but has many other sequences which are never found in the cytoplasm. The phenomenon has been somewhat clarified by the recent discovery of introns in many genes (for a general introduction see ref. 4). Although the evidence is still very preliminary, it certainly suggests that much of the base sequence in the interior of some introns may be junk, in that these sequences drift rapidly in evolution, both in detail and in size. Moreover, the number of introns may differ even in closely related genes, as in the two genes for rat preproinsulin¹⁶. Whether there is junk between genes is unclear but it is noteworthy that the four genes for the human β -like globins, which occur fairly near together in a single stretch of DNA, occupy a region no less than 40 kilobases long¹⁷. This greatly exceeds the total length of the four primary transcripts (that is the four mRNA precursors), an amount estimated to be considerably less than 10 kilobases. There is little evidence to indicate that there are other coding sequences between these genes (although the question is still quite open) and a tenable hypothesis is that much of this interspersed DNA has little specific function.

In summary, then, there is a large amount of evidence which suggests, but does not prove, that much DNA in higher

organisms is little better than junk. We shall assume, for the rest of this article, that this hypothesis is true. We therefore need to explain how such DNA arose in the first place and why it is not speedily eliminated, since, by definition, it contributes little or nothing to the fitness of the organism.

What is selfish DNA?

The theory of natural selection, in its more general formulation, deals with the competition between replicating entities. It shows that, in such a competition, the more efficient replicators increase in number at the expense of their less efficient competitors. After a sufficient time, only the most efficient replicators survive. The idea of selfish DNA is firmly based on this general theory of natural selection, but it deals with selection in an unfamiliar context.

The familiar neo-darwinian theory of natural selection is concerned with the competition between organisms in a population. At the level of molecular genetics it provides an explanation of the spread of 'useful' genes or DNA sequences within a population. Organisms that carry a gene that contributes positively to fitness tend to increase their representation at the expense of organisms lacking that gene. In time, only those organisms that carry the useful gene survive. Natural selection also predicts the spread of a gene or other DNA sequence within a single genome, provided certain conditions are satisfied. If an organism carrying several copies of the sequence is fitter than an organism carrying a single copy, and if mechanisms exist for the multiplication of the relevant sequence, then natural selection must lead to the emergence of a population in which the sequence is represented several times in every genome.

The idea of selfish DNA is different. It is again concerned with the spread of a given DNA within the genome. However, in the case of selfish DNA, the sequence which spreads makes no contribution to the phenotype of the organism, except insofar as it is a slight burden to the cell that contains it. Selfish DNA sequences may be transcribed in some cases and not in others. The spread of selfish DNA sequences within the genome can be compared to the spread of a not-too-harmful parasite within its host.

Mechanisms for DNA spreading

The inheritance of a repeated DNA sequence in a population of eukaryotes clearly requires that the multiplication which produced it occurred in the germ line. Furthermore, any mechanism that can lead to the multiplication of useful DNA will probably lead to the multiplication of selfish DNA (and vice versa). Of course, natural selection subsequently discriminates between multiple sequences of different kinds, but it does not necessarily prevent the multiplication of neutral or harmful sequences.

Multiplication in the germ-line sequence can occur in non-dividing cells or during meiosis and mitosis (within lineages that lead to the germ line). In the former case, the mechanisms available resemble those that are well documented for prokaryotes, that is, multiplication may occur in eukaryotes through the integration of viruses or of elements analogous to transposons and insertion sequences. Doolittle and Sapienza⁵ have discussed these mechanisms in some detail, particularly for prokaryotes. They are likely to lead to the spreading of DNA sequences to widely separated positions on the chromosomes.

During mitosis and meiosis, multiplication (or deletion) is likely to occur by unequal crossing over. This mechanism will often lead to the formation of tandem repeats. It is well documented for the tRNA 'genes' of *Drosophila* and for various other tandemly repeated sequences in higher organisms.

The amount and location of selfish DNA

Natural selection 'within' the genome will favour the indefinite spreading of selfish preferred replicators. Natural selection between genotypes provides a balancing force that attempts to maintain the total amount of selfish DNA at an equilibrium (steady state) level—organisms whose genomes contain an

excessive proportion of selfish DNA would be at a metabolic disadvantage relative to organisms with less selfish DNA, and so would be eliminated by the normal mechanism of natural selection. Excessive spreading of functionless replicators may be considered as a 'cancer' of the genome—the uncontrolled expansion of one segment of the genome would ultimately lead to the extinction of the genotype that permits such expansion. Of course, we do not know whether extinction of genotypes in nature even occurs for this reason.

It is hard to get beyond generalities of this kind. To do so we would, at least, need to know how much selective disadvantage results from the presence of a given amount of useless DNA. Even this minimal information is not easily acquired, so we cannot produce other than qualitative arguments.

It seems certain that the metabolic energy cost of replicating a superfluous short DNA sequence in a genome containing 10^9 base pairs would be very small. If, for example, the selective advantage were equal to the proportion of the genome made up by the extra DNA, a sequence of 1,000 base pairs would produce a selective disadvantage of only 10^{-6} . If the selective disadvantage were proportional to the extra energy cost divided by the total metabolic energy expended per cell per generation, the disadvantage would be much smaller. The selective disadvantage might be greater in more stringent conditions, but it is still hard to believe that a relatively small proportion of selfish DNA could be selected against strongly.

On the other hand, when the total amount of selfish DNA becomes comparable to or greater than that of useful DNA, it seems likely that the selective disadvantage would be significant. We may expect, therefore, that the mechanisms for the formation and deletion of nonspecific DNA will adjust, in each organism, so that the load of DNA is sufficiently small that it can be accommodated without producing a large selective disadvantage. The proportion of nonspecific DNA in any particular organism will thus depend on the lifestyle of the organism, and particularly on its sensitivity to metabolic stress during the most vulnerable part of the life cycle.

We can make one prediction on the basis of energy costs. Selfish DNA will accumulate to a greater extent in non-transcribed regions of the genome than in those that are transcribed. Of course, selfish DNA will in most cases be excluded from translated sequences, because the insertion of amino acids within a protein will almost always have serious consequences, even in diploid organisms (but see the suggestion by F.H.C.C.¹⁸).

At first sight it might seem anomalous that natural selection does not eliminate all selfish DNA. Since the suggestion that much eukaryotic DNA is useless distinguishes the selfish DNA hypothesis from many closely related proposals, it may be useful to take up this point in some detail.

First, the elimination of disadvantaged organisms from a population, by their more favoured competitors, takes a number of generations several times larger than the reciprocal of the selective disadvantage. If the selective disadvantage associated with a stretch of useless DNA in higher organisms is only 10^{-6} , it would take 10^6 – 10^8 years to eliminate it by competition. For typical higher organisms this is a very long time, so the elimination of a particular stretch of selfish DNA may be a very slow process even on a geological time scale. Second, the mechanisms for the deletion of short sequences of DNA may be inefficient, since there is no strong selective pressure for the development of 'corrective' measures when the 'fault' carries a relatively small selective penalty. Taken together, these arguments suggest that the elimination of a particular piece of junk from the genome may be a very slow process.

This in turn suggests that the amount of useless DNA in the genome is a consequence of a dynamic balance. The organism 'attempts' to limit the spread of selfish DNA by controlling the mechanism for gene duplication, but is constrained by imperfections in genetic processes and/or by the need to permit some duplication of advantageous genes. Selfish DNA sequences 'attempt' to subvert these mechanisms and may be able to do so

comparatively rapidly because mutation will affect them directly. On the other hand, the defence mechanisms of the host are likely to depend on the action of protein and therefore may evolve more slowly. Once established within the genome, useless sequences probably have a long 'life expectancy'.

For any particular type of selfish DNA, there is no reason that a steady state should necessarily be reached in evolution. The situation would be continually changing. A particular type of DNA might first spread rather successfully over the chromosomes. The host might then evolve a mechanism which reduced or eliminated further spreading. It might also evolve a method for preferentially deleting it. At the same time, random mutations in the selfish DNA might make it more like ordinary DNA and so, perhaps, less easy to remove. Eventually, these sequences, possibly by now rather remote from those originally introduced, may cease to spread and be slowly eliminated. Meanwhile, other types of selfish DNA may originate, expand and evolve in a similar way.

In short, we may expect a kind of molecular struggle for existence within the DNA of the chromosomes, using the process of natural selection. There is no reason to believe that this is likely to be much simpler or more easy to predict than evolution at any other level. At bottom, the existence of selfish DNA is possible because DNA is a molecule which is replicated very easily and because selfish DNA occurs in an environment in which DNA replication is a necessity. It thus has the opportunity of subverting these essential mechanisms to its own purpose.

The inheritance of selfish DNA

Although the inheritance of selfish DNA will occur mainly within a mendelian framework, it is likely to be different in detail and more complex than simple mendelian inheritance. This is due both to the multiplication mechanisms, which in one way or another will produce repeated copies (see the discussion by Doolittle and Sapienza⁵), and to the fact that these copies are likely to be distributed round the chromosomes rather than being located in a single place in the genome as most normal genes are. For both these reasons, a particular type of selfish DNA is likely to spread more rapidly through a population than would a normal gene with a low selective advantage. It will be even more rapid if selfish DNA can spread horizontally between different individuals in a population, due to viruses or other infectious agents, although it should be remembered that such 'infection' must affect the germ line and not merely the soma. If this initial spread takes place when the additional DNA produced is relatively small in amount, it is unlikely to be seriously hindered by the organism selecting against it. The study of these processes will clearly require a new type of population genetics.

Can selfish DNA acquire a specific function?

It would be surprising if the host organism did not occasionally find some use for particular selfish DNA sequences, especially if there were many different sequences widely distributed over the chromosomes. One obvious use, as repeatedly stressed by Britten and Davidson^{19,20}, would be for control purposes at one level or another. This seems more than plausible.

It has often been argued (see, for example, ref. 21) that for the evolution of complex higher organisms, what is required is not so much the evolution of new proteins as the evolution of new control mechanisms and especially mechanisms which control together sets of genes which previously had been regulated separately. To be useful, a new control sequence on the DNA is likely to be needed in a number of distinct places in the genome. It has rarely been considered how this could be brought about expeditiously by the rather random methods available to natural selection.

A mechanism which scattered, more or less at random, many kinds of repeated sequences in many places in the genome would appear to be rather good for this purpose. Most sets of such sequences would be unlikely to find themselves in the right

combination of places to be useful but, by chance, the members of one particular set might be located so that they could be used to turn on (or turn off) together a set of genes which had never been controlled before in a coordinated way. A next way of doing this would be to use as control sequences not the many identical copies distributed over the genome, but a small subset of these which had mutated away from the master sequence in the same manner.

On this picture, each set of repeated sequences might be 'tested' from time to time in evolution by the production of a control macromolecule (for example, a special protein) to recognize those sequences. If this produced a favourable result, natural selection would confirm and extend the new mechanism. If not, it would be selected against and discarded. Such a process implies that most sets of repeated sequences will never be of use since, on statistical grounds, their members will usually be in unsuitable places.

It thus seems unlikely that all selfish DNA has acquired a special function, especially in those organisms with very high *C* values. Nor do we feel that if one example of a particular sequence acquires a function, all the copies of that sequence will necessarily do so. As selfish DNA is likely to be distributed over the chromosomes in rather a random manner, it seems unlikely that every copy of a potentially useful sequence will be in the right position to function correctly. For example, if a specific sequence within an intron were used to control the act of splicing that intron, a similar sequence in an untranscribed region between genes would obviously not be able to act in this way.

In some circumstances, the sheer bulk of selfish DNA may be used by the organism for its own purpose. That is, the selfish DNA may acquire a nonspecific function which gives the organism a selective advantage. This is the point of view favoured by Cavalier-Smith in a very detailed and suggestive article¹² which the reader should consult. He proposes that excess DNA may be the mechanism the cell uses to slow up development or to make bigger cells. However, we suspect that both slow growth and large cell size could be evolved just as well by other more direct mechanisms. We prefer to think that the organism has tolerated selfish DNA which has arisen because of the latter's own selective pressure.

Thus, some selfish DNA may acquire a useful function and confer a selective advantage on the organism. Using the analogy of parasitism, slightly harmful infestation may ultimately be transformed into a symbiosis. What we would stress is that not all selfish DNA is likely to become useful. Much of it may have no specific function at all. It would be folly in such cases to hunt obsessively for one. To continue our analogy, it is difficult to accept the idea that all human parasites have been selected by human beings for their own advantage.

Life style

The effect of nonspecific DNA on the life style of the organism has been considered by several authors, in particular by Cavalier-Smith¹² and by Hindergardner⁸. We shall not attempt to review all their ideas here but instead will give one example to show the type of argument used.

Bennett²² has brought together the measurements of DNA content for higher herbaceous plants. There is a striking connection between DNA content per cell and the minimum generation time of the plant. In brief, if such an angiosperm has more than 10 pg of DNA per cell, it is unlikely to be an ephemeral (that is, a plant with a short generation time). If it is a diploid and has more than 30 pg of DNA, it is highly likely to be an obligate perennial, rather than an annual or an ephemeral. The converse, however, is not true, there being a fair number of perennials with a DNA content of less than 30 pg and a few with less than 10 pg. A clear picture emerges that if a herbaceous plant has too much DNA it cannot have a short generation time.

This is explained by assuming that the extra DNA needs a bigger nucleus to hold it and that this increases both the size of the cell and the duration of meiosis and generally slows up the development of the plant. An interesting exception is that the

duration of meiosis, is, if anything, shorter for polyploid species than for their diploid ancestors²³. This suggests that it is the ratio of good DNA to junk DNA rather than the total DNA content which influences the duration of meiosis.

An analogous situation may obtain in certain American species of salamander. These often differ considerably in the rapidity of their development and of their life cycles, the tropical species tending to take longer than the more temperate ones. Drs David Wake and Herbert MacGregor (personal communication) tell us that preliminary evidence suggests that species with the longer developmental times often have the higher *C* values. This appears to parallel the situation just described for the herbaceous plants. It remains to be seen if further evidence will continue to support this generalization. (See the interesting paper by Oeldorfe *et al.*²⁵ on 25 species of frogs. They conclude that 'genome size sets a limit beyond which development cannot be accelerated'.)

Testing the theory

The theory of selfish DNA is not so vague that it cannot be tested. We can think of three general ways to do this. In the first place, it is important to know where DNA sequences occur which appear to have little obvious function, whether they are associated with flanking or other sequences of any special sort and how homologous sequences differ in different organisms and in different species, either in sequence or in position on the chromosome. For example, it has recently been shown by Young²⁴ that certain intermediate repetitive sequences in *Drosophila* are often in different chromosomal positions in different strains of the same species.

Second, if the increase of selfish DNA and its movement around the chromosome are not rare events in evolution, it may be feasible to study, in laboratory experiments, the actual molecular mechanisms involved in these processes.

Third, one would hope that a careful study of all the nonspecific effects of extra DNA would give us a better idea of how it affected different aspects of cellular behaviour. In parti-

cular, it is important to discover whether the addition of nonspecific DNA does, in fact, slow down cells metabolically and for what reasons. Such information, together with a careful study of the physiology and life style of related organisms with dissimilar amounts of DNA, should eventually make it possible to explain these differences in a convincing way.

Conclusion

Although it is an old idea that much DNA in higher organisms has no specific function⁸⁻¹², and although it has been suggested before that this nonspecific DNA may rise to levels which are acceptable or even advantageous to an organism^{8,12}, depending on certain features of its life style, we feel that to regard much of this nonspecific DNA as selfish DNA is genuinely different from most earlier proposals. Such a point of view is especially useful in thinking about the dynamic aspects of nonspecific DNA. It directs attention to the mechanisms involved in the spread and evolution of such DNA and it cautions one against looking for a special function for every piece of DNA which drifts rapidly in sequence or in position on the genome.

While proper care should be exercised both in labelling as selfish DNA every piece of DNA whose function is not immediately apparent and in invoking plausible but unproven hypotheses concerning the details of natural selection, the idea seems a useful one to bear in mind when exploring the complexities of the genomes of higher organisms. It could well make sense of many of the puzzles and paradoxes which have arisen over the last 10 or 15 years. The main facts are, at first sight, so odd that only a somewhat unconventional idea is likely to explain them.

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LETTERS

Observation and search for γ rays 1–20 MeV from the Crab, NGC4151, Cyg X-1, Cyg X-3, CG135+1 and 3C273

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Observations of γ rays of 1–20 MeV with the UCR Compton double scatter γ ray telescope are reported for a balloon flight launched from Palestine, Texas, 4.5 GV, at 01.00 UT 29 September 1978. The energy distribution of the total γ rays from the Crab from 1.2 to 20 MeV is measured. Two σ upper limits of 3, 2, 0.6 and 0.4×10^{-4} photons $\text{cm}^{-2} \text{s}^{-1} \text{MeV}^{-1}$ at energies 1.2–3, 3–5, 5–10 and 10–20 MeV, respectively, are found for the Seyfert galaxy NGC4151, the black hole candidate Cyg X-1 and for Cyg X-3. In the same energy intervals, the 2σ upper limits for the nearest and second nearest QSOs CG135+1 and 3C273 are 5, 3, 1 and 0.4×10^{-4} photons $\text{cm}^{-2} \text{s}^{-1} \text{MeV}^{-1}$. These upper limits restrict confirmed γ -ray sources at 1–20 MeV to the Crab and NP0532. Our upper limits for NGC4151 do not support Seyfert galaxies as the source of cosmic diffuse radiation¹.

The University of California UCR Compton double scatter γ -ray telescope measures the energy of the incident γ ray and its scatter angle in the first scintillator. The method of detection, efficiencies and time, angle and energy resolutions have been described previously^{2,3}. Vertical pairs of cells, only, are used in this analysis. Thus, the scatter angle is equal to the zenith angle and the zenith angle of the source is determined for each observed γ ray as a circle on the sky. The sky is divided into circles around the zenith and γ rays are collected into bins in zenith angle and energy. Because of low telescope efficiencies at small angles, $\leq 10^\circ$, and higher backgrounds at large angles $\geq 30^\circ$, we report here only γ rays with zenith angles between 10 and 30° .

Although corrections were made for gain changes in the energy and angle assignments, telescope thresholds are also affected. Therefore, we chose the computer thresholds high enough, 0.3 and 0.9 MeV for S1 and S2, to be above the electronic thresholds throughout most of the flight. Near the beginning of the flight, however, just after reaching the ceiling limit, the computer thresholds were below the actual ones for the small angle scatters and some real γ rays were rejected. The effect is greatest for the $10\text{--}20^\circ$ scatters so we have used no $10\text{--}20^\circ$ data until 30 September 13.00 UT. From 30 September 09.30 UT to flight termination, the residual atmosphere ranged between 4.5 and 3.2 g cm^{-2} . The downward-moving γ -ray background is a combination of atmospheric, cosmic diffuse and neutron-induced γ rays. The cosmic diffuse and neutron-induced backgrounds are nearly independent of height in the atmosphere but we need to correct for the atmospheric γ rays that vary nearly linearly with depth.

The Crab, NGC4151 and 3C273 crossed the zenith meridian at 11.15, 14.75 and 18.15 UT, respectively, 30 September and 1 October; Cyg X-1 and Cyg X-3 transited at 01.45 and 02.30 UT, respectively, 30 September; and CG135+1 transited at 18.00 UT on 1 October as shown in Fig. 1. With few exceptions, observations were made continuously from 09.30 UT on 30 September to 23.00 UT on 1 October.

The count rates for γ rays of 1.2–10 MeV, corrected for altitude variation, were combined into 30-min intervals and plotted in Fig. 1. The data for zenith angles of $10\text{--}20^\circ$ and $20\text{--}30^\circ$ are shown for the duration of the flight at float. As the Earth turns on its axis, a celestial point source passes through the $10\text{--}20^\circ$ and $20\text{--}30^\circ$ telescope zenith angle rings on the sky. The expected shapes of the count rates from a point source are shown by the solid contours.

The background for the Crab has been chosen as an average fit to the data from 30 September, 15.00 UT to 1 October, 07.00 UT and 1 October 16.00 UT to 1 October 23.00 UT. If other sources exist in this region we would be overestimating the background flux and thus underestimating the Crab flux. As our upper limits for other sources is based on this background we would also be overestimating our upper limits for the other sources.

An 8σ increase in count rate is observed in the $10\text{--}20^\circ$ plot and increases of 10σ and 8σ in the $20\text{--}30^\circ$ plot as the Crab transits the zenith meridian.

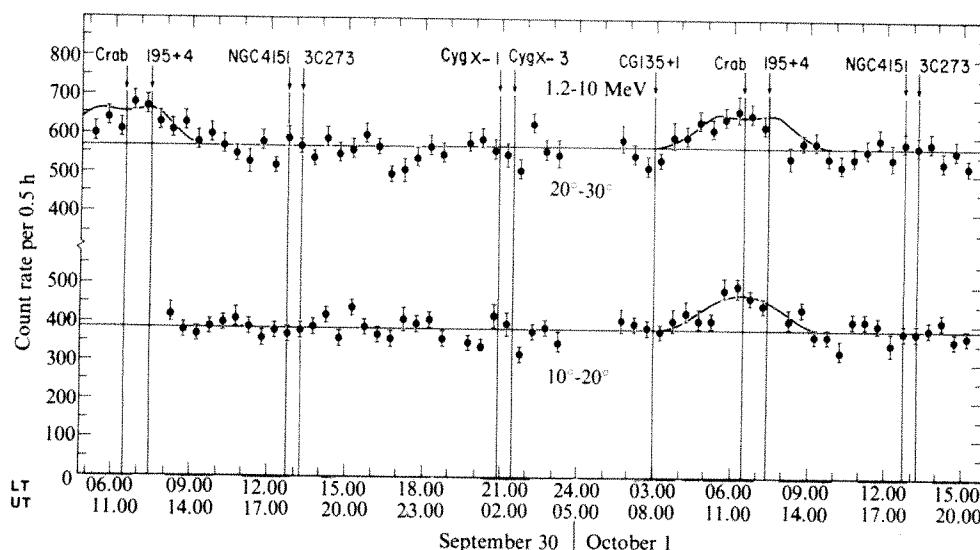


Fig. 1 Counts per 0.5 h versus LT and UT over the flight for γ -ray energies of 1.2–10 MeV and zenith angles of sources of $10\text{--}20^\circ$ and $20\text{--}30^\circ$. Locations of certain possible sources are indicated by arrows. The contours are the shapes of the γ -ray count rate distributions expected from point sources.

The centre and shape of the combined time distribution of the 10–20° and 20–30° data correlate to the counting rate contour expected from the Crab to within 2 degree of arc, with a 1σ uncertainty of 3 degree of arc. This eliminates the SAS 2 source, 195+4 and the two COS B sources in the anti-galactic centre direction, CG176–7 and CG189+1, as the origin of the γ rays.

The telescope, with its finite angular resolution, gives a distribution of zenith angles for a point source. The flux in the observation angle rings of 10–20° and 20–30° is calculated at any desired time and the flux missed from outside these rings is estimated and added. For the Crab, at the time of nearest approach, 9°, at the centre of the contours, the flux from 1.2 to 10 MeV is $3.9 \pm 2.0 \times 10^{-3}$ photons $\text{cm}^{-2} \text{s}^{-1}$.

The energy distribution is determined from plots similar to those in Fig. 1 and plotted in Fig. 2 for the energy intervals of 1.2–3, 3–5, 5–10 and 10–20 MeV. The point at 10–20 MeV gives an upper limit only. Our values are only slightly higher than and compatible with the extrapolations of Walraven *et al.*⁴ and Laros *et al.*⁵ from X-rays to γ -ray energies, as are the recent values of Penningsfeld *et al.*⁶. Our fluxes are about 0.4 of the values of Wilson *et al.*¹¹ measured with the same telescope in 1976 and 0.7 of the preliminary fluxes from this data reported in Kyoto¹. The differences, outside of statistics, arise mostly from the methods of background assignment. Our current values are considered most reliable.

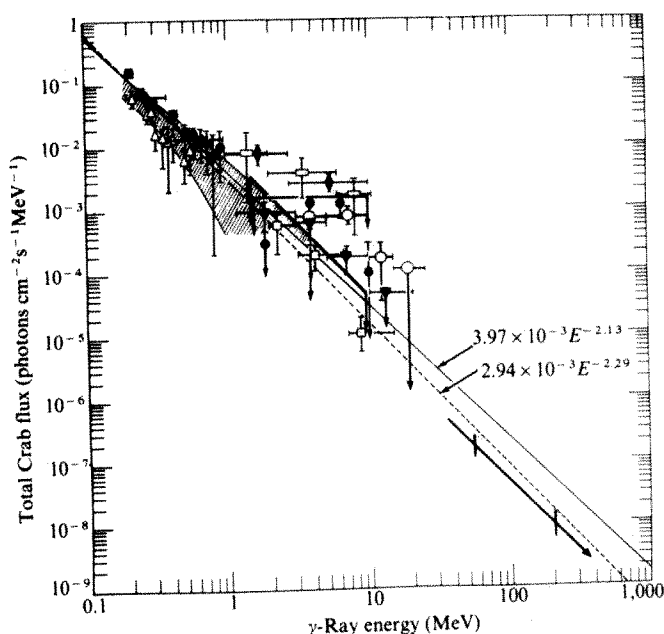


Fig. 2 The energy distribution of X rays and γ rays from the Crab. \blacktriangledown , This experiment; \square , ref. 6; \circ , ref. 11; \triangle , ref. 8; \square , ref. 12; \bullet , ref. 4; \downarrow , ref. 9; \diamond , ref. 7; \uparrow , ref. 10; refs 13, 14, \square , fit of Walraven *et al.*; $---$, fit of Laros *et al.*⁵.

NGC4151 crossed within 8° of the zenith at two times separated by 24 h. There is no evidence in our data from the 10–20° or 20–30° angle intervals for an excess flux at either time. For fluxes from NGC4151 2σ upper limits have been calculated from the background counting rate and are plotted in Fig. 3. These upper limits are factors of 5–10 below the fluxes reported by di Cocco *et al.*¹⁵, Graml *et al.*¹⁶ and Perotti *et al.*¹⁷. The results of Graml *et al.*¹⁶ were withdrawn at the 16th International Cosmic Ray Conference in Kyoto, August 1979, and were replaced by upper limits of 14, 5.5, 2.6 and 0.46×10^{-4} photons $\text{cm}^{-2} \text{s}^{-1} \text{MeV}^{-1}$ at energies of 1.1–2, 2–4, 4–6 and 6–20 MeV, respectively (V. Schönfelder, personal communication). Meegan and Haymes¹⁸ also recently published upper limits for NGC4151. Unless NGC4151 is varying widely in time, by factors of 5–10, our values are in direct conflict with

the above reported fluxes^{15,17} that would have given peaks on Fig. 3 twice as high as the Crab maxima.

As a consequence of our low upper limits and the other upper limits of Fig. 3, the suggestion by Schönfelder²⁶ that the diffuse cosmic γ radiation is the result of superposition of many unresolved galaxies such as the Seyfert galaxy NGC4151 seems premature. There is no observed break in the energy distribution of NGC4151 at 3 MeV and no reason to connect the Seyfert galaxies to the cosmic diffuse γ -ray energy distribution.

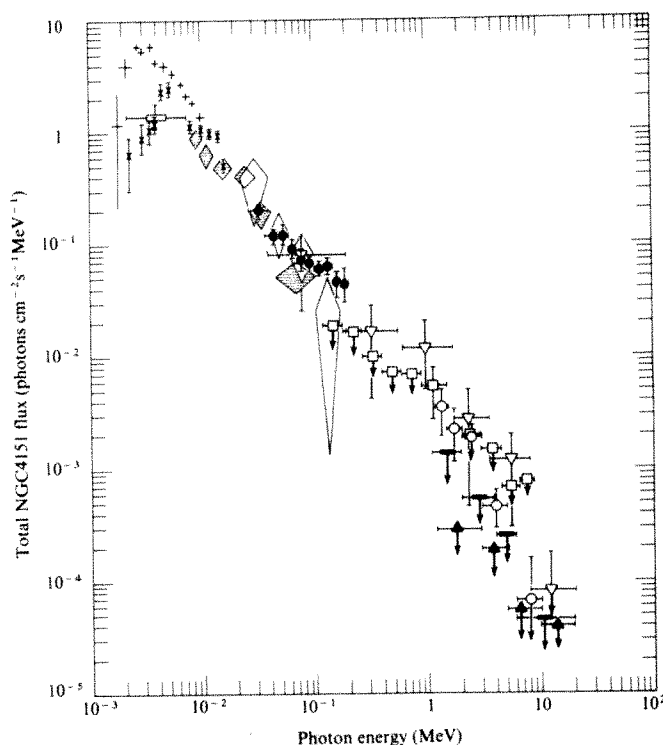


Fig. 3 The energy distribution of X rays and γ rays from NGC4151. \blacktriangle , This experiment; \times , refs 21, 22; \square , ref. 23; \diamond , ref. 20; \diamond , ref. 25; \bullet , ref. 19; $+$, ref. 24; \square , ref. 18; \circ , ref. 16; ∇ , ref. 17; \blacksquare , F. Graml *et al.* personal communication.

Although Cyg X-1 and Cyg X-3 passed nearly overhead no evidence for either is seen in Fig. 1. The 2σ upper limits are the same as for NGC4151: 3, 2, 0.6 and 0.4×10^{-4} photons $\text{cm}^{-2} \text{s}^{-1} \text{MeV}^{-1}$ for the energy intervals 1.2–3, 3–5, 5–10 and 10–20 MeV, respectively. For Cyg X-1 our upper limits are a factor of 50 below the fluxes of Baker *et al.*¹² from 1.2 to 10 MeV. Our upper limit from 1.2 to 3 MeV is at the lower limit of the value of Mandrou *et al.*^{13,14}. The fluxes from Cyg X-1 reported by Baker *et al.*¹² would show up on Fig. 1 as peaks 10 times our Crab maxima. It seems that an unusually high time variation, much greater than observed at lower energies (see, for example, ref. 27) would be required to explain the widely varying results of the different observations.

γ -Ray count rates 5σ above background for energies >150 keV were recently reported from the nearest QSO, CG135+1 (ref. 28); however, no absolute fluxes were given. During our flight CG135+1 transited at a minimum zenith angle of 30.3° and its zenith angle was $<35^\circ$ for 4 h. Somewhat less than half of the count rate is expected in the 10–20° and 20–30° angle intervals. Figure 1 shows no excess γ -ray flux from 1.2 to 10 MeV during this time. Using the same procedure as for the other sources, 2σ upper limits of 5, 3, 1.0 and 0.4×10^{-4} photons $\text{cm}^{-2} \text{s}^{-1} \text{MeV}^{-1}$ at energies of 1.2–3, 3–5, 5–10 and 10–20 MeV are obtained for CG135+1.

γ -Rays of 50–500 MeV from the second nearest QSO, 3C273, were reported from COS B observations²⁹, but no fluxes

have been measured at energies of 1–20 MeV. During our flight, 3C273 twice transited at a minimum zenith angle of 28.8° and was at zenith angles less than 35° for 3 h each time. No excess is seen in Fig. 1 on either transit. We find the same 2σ upper limits for 3C273 as for CG135+1.

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Multi-ion complexes in the stratosphere—implications for trace gases and aerosol

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The fate of stratospheric ions was, until recently, thought to be spontaneous neutralisation following mutual recombination of oppositely charged species. According to Ferguson^{1–3} this need not necessarily apply for sufficiently complex cluster ions, as clustering may stabilise ions against neutralisation. Thus, if neutralisation does not occur, stable ion pairs may be formed. This possibility seemed more likely after the first simultaneous composition measurements of stratospheric positive and negative ions^{4–6} revealed the presence of species more stable than those predicted on theoretical grounds; this led to consideration of a possible role of ion pairs in aerosol formation⁷. Here the formation of ion pairs is investigated in the light of new stratospheric ion composition data. Interactions of ion pairs with other stratospheric constituents, and their significance for aerosol and trace gases is discussed and an experimental check of the existence of the hypothetical stratospheric ion pairs is suggested.

First I consider which ion species among those observed in the stratosphere (positive and negative ions were measured simultaneously around an altitude of 36 km) satisfy the requirements

for stable ion pair formation which is

$$E_D > E_N + E_C \quad (1)$$

Here E_D is the effective energy required to detach the electron from the negative cluster ion, E_N is the effective energy released on neutralisation of the positive cluster ion by a free electron and E_C is the energy released on formation of a chemical bond possibly formed on interaction of core ions or ligands. E_D and E_N are related to the electron affinity E_A of the negative ion core and the ionisation potential IP of the positive ion core by $E_D = E_A + S_-$ and $E_N = IP - S_+$ where S_- and S_+ are the total solvation energies of the negative and the positive cluster ion.

Inspection of the most abundant ions observed around 36 km (Table 1) reveals that the requirement (1) is not fulfilled for the recombination of proton hydrates with NO_3^- (HNO_3)₂, as an E_C of 4.5 eV corresponding to an H–HNO₃ bond⁸ which probably forms has to be considered. Recombination of the observed proton hydrates with HSO_4^- (HNO_3)₂ probably also leads to spontaneous neutralisation as E_D should not be sufficiently large to compensate for the energy E_C corresponding to an H–HSO₄ bond being 4.5 eV (ref. 9). Thus, recombination of the observed proton hydrate ions does not apparently lead to stable ion pairs. Whether or not the observed ions containing the core HX^+ may form stable ion pairs depends on the proton affinity P_A of the as yet not safely identified molecule X. If, as suggested by Ferguson², X was indeed NaOH due to the extremely high P_A of this molecule the HX^+ ions should form stable ion pairs. If, however, X was another molecule having a considerably lower P_A than NaOH only a fraction of the HX^+ -ion recombination may lead to stable ion pairs, depending on the corresponding E_D and E_N and $PA(X)$, although exceeding $PA(\text{H}_2\text{O})$ (ref. 4), may be too low to allow for stable ion pair formation from the observed HX^+ ions.

Note that recombination of more complex cluster ions which have small abundances around 36 km, but which may be even dominant at lower heights should lead to stable pairs even if proton hydrate ions are involved.

In the hypothetical formation of ion pairs, which may be accompanied by loss of some ligand molecules, a stable ion pair interacts with gas molecules. Such interactions may give rise not only to new ligands, but also to a change of core ions. Moreover, due to its large dipole moment the ion pair attaches a free ion quite efficiently. Thus, a three-ion complex may be formed which due to its net charge rapidly attaches an oppositely charged free ion and may form the four-ion complex. Further attachment of free ions may lead to larger multi-ion complexes (MIC) that have the nature of ion crystals or salt particles. An alternative growth mechanism for MIC may be mutual coalescence. Attachment of MIC to pre-existing aerosol particles may also be considered. MIC formation and growth may represent a gas-to-particle formation mechanism which does not require condensation and consequently does not require a supersaturated gaseous species. If, however, a supersaturated species exists, such as sulphuric acid, MIC which have grown to sufficiently large sizes may act as condensation nuclei. The latter have critical sizes which depend on the supersaturation ratio and thus on the abundance of the condensable species and on the temperature. MIC may, therefore, represent a new and interesting class of stratospheric condensation nuclei which had been thought to be mostly of tropospheric and extraterrestrial origin¹⁰. An *in situ* formation of stratospheric condensation nuclei, particularly one initiated by ions was considered to be inefficient^{2,11}. In contrast to the classical mechanism for ion-induced nucleation which requires individual cluster ions to grow to critical sizes and which is inefficient in the stratosphere mainly because there is no gas which is sufficiently supersaturated and is abundant enough to allow for a rapid growth of ions before these recombine. The MIC mechanism avoids this problem by forming condensation nuclei by coalescence of subcritically sized cluster ions. Apart from its possible influence

Table 1 The most abundant positive and negative ions measured around 36 km altitude^{6,16} with their fractional abundances

Positive ion	Abundance (%)	E_N (eV)	Negative ion	Abundance (%)	E_D (eV)
$H^+(H_2O)_4$	21.3	4.03	$NO_3^-(HNO_3)_2$	65.6	5.86
$H^+(H_2O)_5$	17.8	3.38	$HSO_4^-(HNO_3)_2$	14.2	>6.45
$Na^+(H_2O)_4$	19.3	1.92			
$HX^+X(H_2O)_2$	10.7	(<2.53)			
$HX^+X(H_2O)_3$	16.0	(<1.92)			

Values for E_N and E_D are based on measured^{3,17} and estimated cluster ion bond energies. It was assumed that X is NaOH and that NaOH bonds to Na^+ at least as strongly as H_2O and that HNO_3 bonds to HSO_4^- at least as strongly as it bonds to NO_3^- . A measured lower limit to the electron affinity of HSO_4^- (4.5 eV) (ref. 7) was taken. The electron affinity of NO_3^- was taken as 3.9 eV (ref. 18).

on aerosol formation MIC may also have an impact on stratospheric trace gases which may not only condense but also undergo reactions on MIC surfaces. Trace gases which react with, or attach to, these ions may be irreversibly removed from the stratosphere when ions become incorporated into MIC and sediment out of the stratosphere. One example may be sulphuric acid which was found to have a very low gas phase abundance in the stratosphere and which was suspected to be removed by ion processes¹².

If aerosol formation through MIC were significant, it would provide a link between solar activity which controls the stratospheric and tropospheric ionisation sources (galactic and solar cosmic radiation) and stratospheric and possibly also upper tropospheric aerosol. According to Dickinson¹³ such a link seems to be the most favourable way of connecting the Earth's climate to solar activity.

The volume rate R_V at which gas is converted into aerosol by the MIC process is $R_V = \alpha n^2 AM$. Here, α , n and M are the ion-ion recombination coefficient, the total number densities for free positive and negative ions and the average mass of an ion pair, respectively. The parameter A which ranges between 0 and 1 describes the fraction of recombinations which lead to stable ion pairs. Assuming X to be NaOH A becomes 0.5 at 36 km and thus M becomes 4.7×10^{-22} g if α and n are taken as 10^{-7} cm³ s⁻¹ and 2,000 cm⁻³ (ref. 6). For the evaluation of M it was assumed that the mass of an ion pair equals the sum of the masses of the two free cluster ions which mutually recombine. Thus, R_V becomes 1.2×10^{-22} g cm⁻³ s⁻¹. Integration of R_V over the height interval 55–33 km where 'non proton hydrates' have been observed^{4,6} yields a column rate for gas to particle conversion $R_C = 6.3 \times 10^{-17}$ g cm⁻² s⁻¹. Here A was taken to be equal to the fractional abundance of 'non-proton hydrates'⁴ and n_+ and n_- were computed from a mid latitude galactic cosmic ray ionisation rate considering α -values as given by Smith and Church¹⁴. When compared with the column rate for gas-to-particle conversion required to account for the observed stratospheric aerosol layer which is estimated to be about 1.1×10^{-15} g cm⁻² s⁻¹ (ref. 15) the above value is much smaller.

Thus, MIC formation and growth in the upper stratosphere followed by sedimentation of larger MIC into the region of the main aerosol layer apparently cannot account for the total aerosol mass observed in this layer. If, however, at heights below 33 km all ions had the potential to form stable ion pairs ($A = 1$), the MIC process may even account for a large fraction of the total aerosol mass observed.

To estimate MIC abundances it is assumed that ion pairs (2-IC) are formed by mutual recombination and are lost by free ion attachment (rate constant k assumed to be of the order of 10^{-9} cm³ s⁻¹—the upper limit for an ion molecule reaction) to aerosol particles (time constant τ ; a sticking coefficient of 1 is assumed) a steady state treatment yields

$$n_2 = \frac{A \alpha n^2}{k 2n + \tau^{-1}} \quad (2)$$

and

$$n_3^+ = A n \left(2 + \frac{1}{k n \tau} \right)^{-1} \quad (3)$$

where n_2 and n_3 are the total number densities of 2-IC and positive 3-IC. Neglecting aerosol interaction for an altitude around 35 km ($A = 0.5$, $n = 2,000$ cm⁻³ and $\alpha = 10^{-7}$ cm³ s⁻¹) n_2 and n_3^+ become 5×10^4 cm⁻³ and 5×10^2 cm⁻³. Analogous steady state considerations lead to $n_4 = 5 \times 10^4$ cm⁻³ and $n_5^+ = 5 \times 10^2$ cm⁻³. A more realistic estimate including aerosol interaction with $\tau = 10^5$ s (ref. 12) yields $n_2 = 5 \times 10^4$ cm⁻³, $n_3^+ = 1.3 \times 10^2$ cm⁻³, $n_4 = 2.9 \times 10^1$ cm⁻³ and $n_5^+ = 2.9 \times 10^{-1}$ cm⁻³. For negatively charged MIC the number densities should be the same as for positive ones. Thus, for the more realistic case, charged MIC apparently contribute a few per cent to the total concentration of charged particles. Also, the abundance of charged MIC apparently decreases steeply with increasing number of ions contained in these complexes. The estimates, however, depend sensitively on τ and thereby on the surface area density of the aerosol, the value of which is somewhat uncertain.

The present detection limit of balloon-borne ion mass spectrometers is ~ 1 particle cm⁻³ for both positively and negatively charged species. When compared with its value, the above estimate of $n_3^+ = n_3^-$ is much larger and, consequently, charged MIC should be detectable even if several individual species of 3-IC are present, and their partial number densities are therefore, considerably smaller than their total number density. Previous instruments were probably not suitable for detection of charged MIC due to their low mass ranges.

Therefore, I suggest a search for stratospheric charged MIC, using ion mass spectrometers with greatly increased mass range. This may provide information relevant to stratospheric aerosol- and trace-gas processes and may be the most promising way of obtaining experimental information about the hypothetical MIC that are difficult to study in the laboratory.

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Jointed blocks of peridotite xenoliths in basalts and mantle dynamics

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In considering the formation of fracture in the upper mantle of the Earth, several authors^{1,2} have pointed out that the major problem in the formation of cracks is the fact that tensile stresses in the upper mantle region, particularly in the lithospheric plate, are too small compared with the hydrostatic overburden pressure. From theoretical considerations, Anderson² demonstrated that cracks can nucleate in the lithospheric plates by the accumulation of low density fluid at the base of the plate in the presence of tensile stresses parallel to the plate surface. However, there has been no direct geological observation that supports this conclusion. This letter reports geological observations of planar features in ultramafic inclusions which may have important implications on the existence of fracture in an ambient upper mantle condition.

Ultramafic inclusions in alkalic and related basalts provide much information about the Earth's upper mantle. These inclusions, mostly spinel and garnet peridotites, are considered to be the broken pieces of the wall rocks of the volcanic conduits. In this way, these rocks are the natural 'drill-hole' samples of the upper mantle and may come from depths of up to 200 km (refs 3, 4). Numerous aspects of these rocks have been studied, including the major and minor element chemistry, the isotopic chemistry, the general petrology and petrography, and the rheological properties, to understand the composition and evolution of the upper mantle. However, very little is known about the process by which these inclusions are torn off the walls of the volcanic conduits. The process apparently indicates brittle failure in the mantle in the immediate vicinity of hot magma where the temperatures, in contrast, are appropriate for plastic behaviour.

During petrologic investigation of the ultramafic xenoliths in basalts from San Quintin, Baja California⁵, I observed that many of the xenoliths show angular to subangular shapes resulting from more than one bounding planar surfaces. In some xenoliths three orthogonal sets of such planar surfaces can be recognised. Wilshire and Trask⁶ also recognised similar features in xenoliths from Dish Hill, California. Therefore, planar surfaces in xenoliths in basalts may be common but generally disregarded.

Figure 1 shows a spinel lherzolite xenolith from San Quintin with planar features. The xenolith has a tabular shape, with one perfectly flat surface which measures 25 cm diagonally. Approximately 4 cm away from this surface is another planar feature—a joint plane—that cuts across the massive xenolith. All the bounding surfaces of the xenolith are covered by the frothy, alkalic basalt. As the joint plane does not cut across the host lava, it must have formed before the consolidation of the latter. This observation also suggests that the commonly observed angular to subangular xenoliths result from the formation of sets of joint planes in the upper mantle before invasion by the basalts that carried the xenoliths to the surface. Once the xenoliths are included in this basalt, they are brought up to the surface relatively rapidly by the host basalt as discussed in the following section.

The maximum time spent by the xenoliths in host basalts during the ascent of the magma can be estimated by using Stokes' Law, which states that

$$V_s = \frac{2\Delta\rho ga^2}{9\eta}$$

where V_s is the velocity of settling of a spherical solid of radius a ; $\Delta\rho$, the difference in density between the solid and the fluid; η , the viscosity of the fluid; and g the acceleration due to gravity. Using $a = 15$ cm, the radius of the largest xenolith found in San Quintin, $\Delta\rho = 0.6$ (density of xenolith 3.3 g cm^{-3} and that of basaltic glass 2.7 g cm^{-3}) and $\eta = 10^3 \text{ P}$ for alkalic basalt, we obtain $V_s = 30 \text{ cm s}^{-1}$. Therefore, in a static alkali basaltic magma chamber the xenoliths will settle with as high a velocity as 30 cm s^{-1} . For the magmas to ascend to the surface containing the xenoliths, they must ascend with a velocity $> 30 \text{ cm s}^{-1}$. The depths from which the xenoliths come may be as deep as 75 km. Therefore, with a velocity of ascent of 30 cm s^{-1} , the basalt will take 70 h or ~ 3 days to arrive at the surface from a depth of 75 km. As 30 cm s^{-1} is the minimum velocity ascribed to the ascending magma, 3 days is the maximum time the magma takes to arrive at the surface. Therefore, the xenoliths could not have spent more than 3 days in the ascending basalt. An important parameter in the above estimate is the viscosity of alkali basalt taken as 10^3 P . Recent experimental determination⁷ of this parameter suggests that 10^3 P may be higher than the actual viscosity measured under high pressure. In that case, the mean residence time of xenoliths in liquid basalt must be < 3 days. This determination of the relatively short residence time of xenoliths in their host basalts is important because it justifies the interpretation of the chemistry, mineralogy, texture and structure of the xenoliths as representative of an equilibrium condition in the upper mantle.

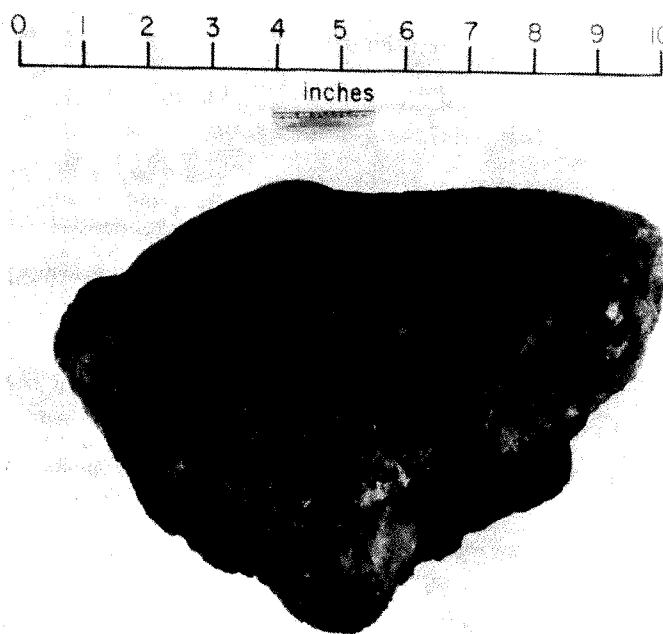


Fig. 1 A jointed block of spinel lherzolite inclusion in alkalic basalt showing a penetrative joint plane parallel to the black marker line. The block of lherzolite is surrounded on all sides by the frothy, scoreaceous basalt that brought up the inclusion to the surface.

There are two possible interpretations for the origin of the joint planes in the xenoliths. First, they could have formed because of the sudden release of lithostatic pressure during the rapid transport to the surface. If that is true, then the joint planes must represent pre-existing planes of weakness. Second, the joints formed by brittle failure in the upper mantle before the xenolith's incorporation by the magma. Note in this connection the mode of occurrence of amphibole selvages on flat surfaces in some xenoliths from Dish Hill, California⁶. The occurrence of amphibole selvages on flat surfaces of these peridotites may indicate that they formed as veins along joint planes in upper mantle conditions.

Is it possible for joint planes to develop under high lithostatic pressure in the upper mantle? It is well known that the presence

of fluid within a massive body of rock reduces the strength of the rock^{8,9}. In effect, the fluid pressure reduces the load pressure, thereby reducing the strength of the rock⁸. This principle has been applied to the effective decrease in strength of a rock due to the generation of magma and to the process of crack propagation by magma in the brittle failure region¹¹. The field of brittle fracture is expanded by fluid pressure because the pressure of the fluid in pores and cracks counteracts the normal stress across the cracks from lithostatic pressure. However, there is no apparent evidence for the presence of fluid magma or volatiles along the joint plane of the lherzolite xenolith under discussion. In the absence of the evidence of such fluid phases along the joint plane, the alternative and most viable explanation for the origin of the joint planes is that they formed under high differential stress of the order of several kilobars.

Analysis of stresses in ultramafic xenoliths showing varying degrees of plastic deformation indicate a range of 250 to 700 bar and analyses of upper mantle strain rates show that rates of 10^{-15} to 10^{-13} s^{-1} are typical for plastically deformed mantle as represented by ultramafic xenoliths¹². However, the joint planes in the xenolith under discussion must have formed at much higher stresses and the corresponding strain rates must be significantly higher than 10^{-13} s^{-1} .

An argument can be made against the interpretation that the joint planes in the xenolith formed due to the release of lithostatic pressure. During the transport of the xenoliths to the surface from mantle depths, two competing processes will operate: decompression due to pressure release and the contraction due to fall in temperature. The anisotropy of olivine, the major constituent of the xenoliths, is of the order of 5% (ref. 13). Thus, the low differential compressibility of olivine in the xenolith is expected to be manifest in the scale of the individual grains. The net effect of these processes can be observed in the mineral grains of many xenoliths which become uncompact due, possibly, to this differential compressibility. The scale of the observed joint planes (several centimetres) in the xenolith (Fig. 1) indicate then the presence of directed stress of at least a similar scale.

Therefore, it is possible that the joint plane (Fig. 1) in the lherzolite xenolith formed in the upper mantle by brittle failure; some intermediate focus earthquakes such as those in Hawaii¹⁴ may indicate this brittle behaviour. When the alkalic magma rises from below, it picks up the jointed blocks of the mantle and brings them to the surface relatively rapidly. In this way, the jointed blocks of the xenoliths will still preserve the ambient properties of the upper mantle, such as high P , T conditions of mineralogical equilibria and evidence of plastic flow.

The most probable explanation for the origin of the joint plane in the lherzolite (in Fig. 1) is that it formed because of high differential stress in the upper mantle. The commonly observed angular to subangular shapes of mantle-derived xenoliths in alkali basalt reflect this phenomenon, and provide a mechanism for these mantle rocks to be torn off the walls of the volcanic conduits.

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Uppermost Miocene carbon isotope stratigraphy of a piston core in the equatorial Pacific

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Carbon isotopic analyses of benthonic foraminifera from palaeomagnetically dated piston core RC12-66 (east equatorial Pacific Ocean) show a permanent decrease in $\delta^{13}\text{C}$ of $\sim 0.5\%$ in the upper reversed interval of palaeomagnetic Epoch 6 (about 6.3 Myr BP). Magnetostratigraphic and biostratigraphic correlations reported here suggest that this decrease is isochronous with a similar carbon shift observed at localities elsewhere in the Indo-Pacific region.

A permanent decrease of $\sim 0.5\text{--}0.8\%$ in the $\delta^{13}\text{C}$ of benthonic foraminiferal calcite has been reported from the Pacific¹⁻⁴ and Indian Oceans⁵ during the late Miocene. These studies are part of the CENOP (Cenozoic Palaeoceanography) programme which is seeking to define isochronous markers for palaeoceanographic reconstructions⁶. This shift in $\delta^{13}\text{C}$ has been interpreted as an oceanwide change in the metabolic CO_2 content of deep waters, resulting from a change in circulation and upwelling patterns, from increased supply of organic matter to the deep sea due to a late Miocene regression, and from a change in ocean fertility^{2,5,7}. Palaeomagnetic dating of this event at an uplifted shallow-marine sequence (Blind River) in New

Table 1 Carbon isotopic composition of *G. subglobosa*, core RC12-66

Depth (cm)	$\delta^{13}\text{C}$ (% PDB)	Depth (cm)	$\delta^{13}\text{C}$ (% PDB)
1,730	-0.57	2,195	-0.95
1,870	-0.79	2,237	-0.60
1,875	-1.00	2,299	-0.56
1,877.5	-0.91	2,319	-0.91
1,887.5	-1.03	2,352	-0.82
1,890	-0.76	2,359	-0.62
1,915	-0.73	2,380	-1.08
1,962.5	-0.46	2,399	-0.89
1,975	-1.01	2,419	-0.66
1,985	-0.43	2,439	-1.02
2,015	-0.36	2,445	-0.98
2,027	-0.55	2,458	-0.63
2,050	-0.79	2,462	-0.30
2,062.5	-0.73	2,478	-0.64
2,077.5	-0.71	2,499	-0.70
2,085	-0.61	2,519	-0.30
2,092.5	-0.65	2,535	-0.78
2,102.5	-0.57	2,539	-0.23
2,125	-0.97	2,559	-0.59
2,135	-0.51	2,560	-0.70
2,147	-0.55	2,579	-0.73
2,155	-0.98	2,598	-0.13
2,165	-0.91	2,602	-0.38
2,175	-0.72	2,627	-0.31
2,185	-0.55	2,690	-0.21
		2,782	-0.22

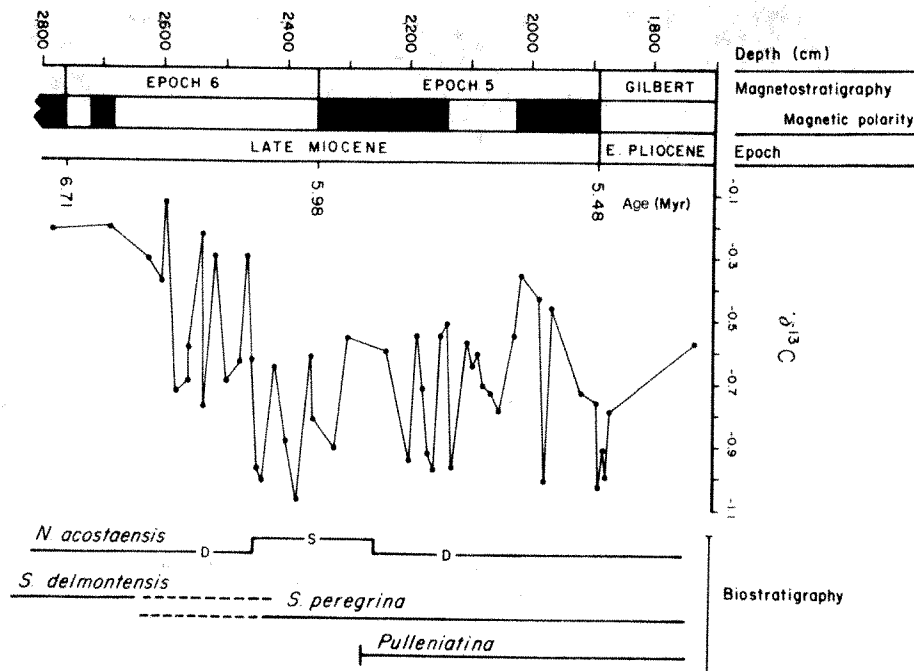


Fig. 1 Carbon isotopic results (‰, PDB), magnetostratigraphy¹⁰, and biostratigraphy¹¹ of the latest Miocene at core RC12-66.

Zealand places it at ~6.2 Myr, within palaeomagnetic Epoch 6 (refs. 3, 8, 9). It has still not been proved that the late Miocene carbon shift represents an isochronous event because it has been palaeomagnetically dated only at the Blind River section. From biostratigraphic evidence, however, the late Miocene carbon shift was assigned ages of ~6.5 Myr (ref. 1) and ~6.2 Myr (ref. 5).

We have found the carbon shift in east Equatorial Pacific Ocean piston core RC12-66. Core RC12-66 (02°36.6' N, 148°12.8' W, 4755m) is one of the longest piston cores recovered from the deep ocean (28 m). This core has been the subject of detailed magnetostratigraphic¹⁰ and biostratigraphic studies^{11,12}. Magnetic plug samples (of ~5 cm³) originally studied by Foster and Opdyke¹⁰ were provided by L. H. Burckle of Lamont-Doherty Geological Observatory. The samples were washed and specimens of the benthonic foraminifer *Globocassidulina subglobosa* were picked from the size fraction greater than 175 µm and analysed using standard procedures¹³.

Carbon isotopic results (Table 1), expressed as $\delta^{13}\text{C}$ with respect to the standard PDB, decrease upwards by about 0.5‰ between ~2,450 and 2,600 cm (Fig. 1). There is unexplained high frequency variability of as much as 0.6‰, but on average the shift seems permanent. Pliocene and Pleistocene $\delta^{13}\text{C}$ results from nearby piston core V28-179 (ref. 14) are on average lighter than the lowest results at RC12-66. Likewise, other sections in the Indo-Pacific show that this is indeed a permanent change.

The age that is applied to the 1.5-m interval over which the shift occurs depends on the age assignment of the palaeomagnetic boundaries of magnetic Epoch 6. Both this study and that of Loutit and Kennett use a new time scale from the Gilbert/Gauss boundary to the inferred base of Epoch 6 based on direct dating of subaerially erupted lavas in Iceland¹⁵. Ages for the boundaries of Epoch 6 given by McDougall *et al.*¹⁵ and corrected in accordance with Mankinen and Dalrymple¹⁶ are ~5.98 and 6.71 Myr. Using this time scale, which is probably the most accurate for magnetic Epoch 6, we estimate the age of the late Miocene carbon shift to be ~6.3 Myr. The corrected time scale of McDougall *et al.*¹⁵ indicates an age of ~6.2 Myr for the carbon shift in the Blind River section³, in good agreement with that for RC12-66. Magnetostratigraphy, therefore, suggests that the carbon shift is isochronous between the south-west Pacific land-based sequence and the east equatorial Pacific Ocean.

Biostratigraphy also suggests the carbon shift is an isochronous marker in deep-sea sequences. At RC12-66 the carbon shift interval is marked by the evolution of the radiolarian *Stichocorys peregrina* from *Stichocorys delmontensis*¹¹. Just above this level there is an interval of sinistral *Neoglobobulimina acostaensis*, followed by the first appearance of *Pulleniatina primalis*. This is the same sequence of events found at DSDP 158 (ref. 1). In addition, where nanofossil biostratigraphy has been examined, it is found that the carbon shift is associated with the first appearance of *Ammaurolithus primus*¹⁷.

At DSDP Site 158 the carbon shift was originally reported to occur within a 3-m interval¹, but subsequent more detailed studies by one of us (L.D.K.) reveal that it is restricted to only a 1-m interval. The sedimentation rate through this interval is estimated to be ~50m Myr⁻¹ (ref. 18), and thus the carbon shift seems to have occurred in ~50,000 yr. In the New Zealand Blind River section an upper limit on the duration of the shift is 150,000 yr. (ref. 3). At RC12-66 we estimate from the magnetostratigraphy of Foster and Opdyke¹⁰, that the late Miocene sedimentation rate is ~8m Myr⁻¹. This rate provides a duration of this event of ~200,000 yr at RC12-66. Further studies of high sedimentation rate cores are necessary to establish the minimum duration of this event.

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North Atlantic oceanography as possible cause of Antarctic glaciation and eutrophication

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Tarling¹ suggested that ice ages may occur when continental blocks occupy polar positions, isolating the pole from oceanic influences, and when nearby seas are available to provide moisture for the build-up and maintenance of polar ice caps. Such conditions gradually became established in the Southern Hemisphere where the present ice age began 13 Myr ago². The Cenozoic circum-Antarctic geographic and oceanographic evolution has been recently summarised by Kennett³. I report here that circum-Antarctic palaeogeography and palaeoceanography alone were insufficient to lead to the Antarctic glaciation. A model is presented that suggests that the final triggering events took place in the North Atlantic.

Formation of the Atlantic and Indian oceans during the Cretaceous and the early Cenozoic left Antarctica and Australia in a polar position⁴. Separation of Australia from Antarctica during the late Eocene opened a seaway in which an almost continuous circum-Antarctic current system could form, which in turn effectively isolated the Antarctic continent⁵. Concomitant with, and perhaps caused by this change of ocean configuration was a decrease of global warmth from a mid-Cretaceous high⁶, so that by late Eocene/earliest Oligocene time freezing temperatures prevailed along Antarctic coasts⁷. Although the southern ocean has probably been the principal source of the world oceans' deep and bottom water since at least the late Cretaceous⁸, the formation of Antarctic bottom water at freezing temperatures near the Eocene/Oligocene boundary stimulated the oceans' deep water circulation as manifested by widespread erosional hiatuses⁹, a drastic drop of the carbonate compensation depth^{10,11}, and a reorganisation of benthic deep water faunas^{12,13}. During the remainder of the Palaeogene Antarctica remained a cold but largely ice-free continent.

Zonal winds (polar easterlies, prevailing westerlies), driven by the newly established thermal contrast, set up zonal ocean currents. After opening of the Drake passage near the Oligocene/Miocene boundary, ~22.5 Myr ago¹⁴, a fully circum-Antarctic current formed. The Ekman drift that resulted from these zonal wind fields caused upwelling around Antarctica. This upwelling, however, could have brought up only cold water of low salinity from shallow (~100 m) depth (A. L. Gordon, personal communication). A probable consequence of this upwelling and of the interaction of the vigorous currents with bottom and coastal features is a zone of high biological productivity, noted to have occurred from the late Eocene onward, in proximity to the Antarctic continent¹⁵⁻¹⁷. But in general, the southern ocean remained an oligotrophic region of general downwelling, dominated by calcareous floras and faunas. The great transition to essentially modern conditions, that is Antarctic continental glaciation and circum-Antarctic oceanic eutrophication, occurred during the early and middle Miocene. These events have no apparent 'cause' in any Southern Hemisphere tectonic or oceanographic event, but seem to correlate well with two tectonic events in the Northern Hemisphere that fundamentally rearranged the North Atlantic circulation system: (1) the closure of the Mediterranean at its eastern end, and (2) the subsidence of the Iceland ridge (Greenland-Iceland-Faeroe ridge).

Closure of the eastern Mediterranean during the early Miocene was completed ~18 Myr ago¹⁸, interrupting the circum-

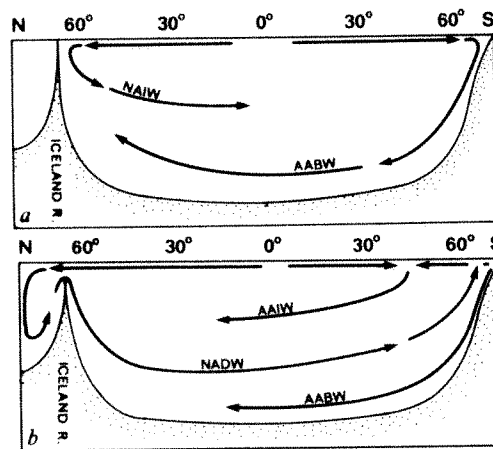


Fig. 1 General current system of the Atlantic Ocean. *a*, Pre-Miocene, with emergent Iceland ridge. *b*, Post-mid-Miocene, with submerged Iceland ridge and Norwegian Sea overflow.

global, near-equatorial flow through Tethys. The resulting sea, lying at or below 30° N latitude⁴, became an evaporation basin which must have contributed increasing amounts of warm and very saline water to the North Atlantic⁸. During the early Miocene the Atlantic probably became, as it still is today, the saltiest of all oceans.

Since seafloor spreading between Greenland and Europe began, ~53 Myr ago, the aseismic Iceland ridge had been an effective barrier between the Atlantic ocean and the north polar sea. During the latest Oligocene, ~25 Myr ago, the first segments of the Iceland ridge began to subside below sea level^{19,20}. This initial subsidence allowed ingress of North Atlantic surface water into the then already cold Norwegian sea²¹, but the barrier was probably still too shallow to allow return flow of deep water into the North Atlantic. By the mid-Miocene, subsidence of the Iceland ridge had progressed to such depths that reflux of cold deep water became significant, thus linking the north polar sea as a heat sink to the world ocean. The establishment of the North Atlantic as a major source of deep water for the world ocean brought about a fundamental rearrangement of the global deep-water circulation.

The early Norwegian Sea overflow water, formed through cooling of North Atlantic water, might not have been very cold, having achieved sufficient density for sinking and outflow as a result of its high salinity which it inherited from the Mediterranean outflow. The outflow of this early Norwegian Sea overflow water soon reached great proportions, causing major sediment redistribution within the North and South Atlantic ocean²²⁻²⁴.

The early North Atlantic deep water which resulted from the Norwegian Sea overflow water, like its modern counterpart, travelled the length of the Atlantic Ocean and inserted itself from below as an intermediate water mass into the circum-Antarctic system. The arrival of this North Atlantic deep water destabilised the vertical structure of the circum-Antarctic system so that the existing wind-induced belt of upwelling could bring North Atlantic deep water to the surface instead of the cold, low salinity local water from shallow depths (A. L. Gordon, personal communication). The upwelling water was nutrient-rich and led to the transformation of an oligotrophic area to one of high biological productivity, which has characterised Antarctic waters until today.

The great volume of relatively warm but saline North Atlantic deep water that was thus injected into the cold circum-Antarctic environment contained heat that could be converted to latent heat by evaporation. The ensuing high evaporation rates supplied much moisture to the Antarctic continent, which had been 'precooled' to below freezing temperatures for nearly 25 Myr, since the late Eocene. Retention of this moisture on Antarctica formed and maintained the continental ice cap. This

sudden build-up of the Antarctic ice cap as soon as sufficient moisture became available suggests that the previous lack of substantial glaciation on Antarctica was probably due to severe dryness rather than moderate temperatures.

Increased thermal contrast in the Antarctic induced stronger, wind fields which led to an enlargement of the upwelling system, as indicated by the progressive northward displacement of the Antarctic convergence³ and also to continued growth of the Antarctic ice sheet, which by the end of the Miocene may have been 50% larger than today²⁵. The eustatic lowering of sea level which accompanied the growth of the Antarctic ice sheet was sufficient to bring about the final separation of the Mediterranean from the Atlantic, the Messinian salinity crisis²⁶. The Mediterranean, therefore, ceased to supply saline water to the North Atlantic. North Atlantic surface water now flowing into the Norwegian-north polar sea required, because of its lessened

salinity, more intensive cooling to achieve sufficient density for sinking and later overflow. The eustatically lowered sea level also shoaled the Iceland ridge and probably caused a reduction of the volume of outflow of this new, colder, and less saline Norwegian Sea overflow water. When this new North Atlantic deep water was brought to the surface by the circum-Antarctic upwelling system it maintained, because of its high nutrient content, the very productive Antarctic siliceous bioprovince, but because of its lower temperature reduced the rates of evaporation from the ocean. Precipitation 'starvation' led to a drastic reduction of the Antarctic ice sheet soon after the Miocene-Pliocene boundary^{27,28}.

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***In vitro* effects of high molecular weight forms of ACTH on the fetal sheep adrenal**

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The direct involvement of the pituitary-adrenal axis in birth has been well established, at least in sheep, and its removal prolongs pregnancy^{1,2}. As part of the process the fetal sheep adrenal grows rapidly during the 10-15 d prepartum and is associated with a large rise in the plasma corticosteroid concentration^{3,4}. This does not seem to result from an increased ACTH secretion^{5,6}. The fetal adrenal *in vivo* seems refractory to circulating ACTH and shows poor response to elevation of plasma concentration^{1,2,7-9}. Thus the signal for the adrenal hypertrophy and the initiation of parturition remains unclear. The responsiveness of the fetal adrenal to ACTH has been re-examined using isolated adrenal cells. The study shows that in the fetal sheep these are not inherently unresponsive to ACTH, but that high-molecular-weight forms of ACTH block the action of ACTH₁₋₃₉. These peptides may be responsible for controlling the activity of the adrenal *in situ*.

To see whether the pathway for ACTH stimulation of steroidogenesis was poorly developed in the fetal sheep adrenal 10-30 d before birth cells were prepared from the gland and incubated *in vitro*. Cells from fetal sheep of 120-135 d were prepared by collagenase digestion^{10,11}. Adrenals were trimmed, weighed, finely chopped with a scalpel blade and suspended in

10 ml of Krebs bicarbonate buffer containing 20 mM-HEPES pH 7.4, 0.5% (w/v) bovine serum albumin and 25 mg of collagenase (Sigma, Type 1) in a 20-ml plastic vial. This was gassed with 95% O₂ and 5% CO₂ at 37 °C for 60 min and the cell suspension stirred with a polytetrafluorethylene paddle rotating at 500 r.p.m. The suspension was filtered through a 100-μm nylon mesh and centrifuged at 200g for 15 min. The cells were washed twice in 10 ml of Krebs bicarbonate buffer containing 0.5 mg ml⁻¹ soya bean trypsin inhibitor (Sigma), then finally suspended in 50 ml of the same buffer containing the trypsin inhibitor. Aliquots of 0.5 ml of this cell suspension were added to 0.1 ml of 50 mM sodium phosphate (pH 7.5) containing a range of ACTH concentrations in 2-ml polystyrene tubes, then incubated under 95% O₂ and 5% CO₂ for 2 h at 37 °C in a shaking water bath. At the end of the incubation the cells were centrifuged at 1,000g for 15 min at 4 °C and the supernatant was used for the assay of cortisol. Details of the collection of plasma samples and of the assay of ACTH and cortisol have been described previously⁷. The ACTH₁₋₃₉ used in these studies was the human standard obtained from the National Institute for Biological Standards and Control, London.

For the preparation of large molecular weight peptides, pituitaries were collected from adult Welsh mountain sheep. They were rapidly frozen in liquid nitrogen and stored at -70 °C. Before chromatography they were thawed and homogenised in 0.02 M-HCl (10 mg in 0.5 ml) with 1 mM disopropylphosphorofluoridate. After centrifugation at 2,500 r.p.m. for 10 min, 0.5-2 ml of the supernatant was run on a 1.6 × 100 cm column of Sephadex G-100 Superfine and eluted with 1 M acetic acid at 1.6 ml per h and 4 °C. Fractions were collected every 15 min. Three large molecular weight peptides A, B and C, which react with anti-ACTH₁₋₂₄, were separated¹². They have approximate molecular weights of 50,000, 30,000 and 20,000 respectively¹². In some cell incubations they were added either in place of ACTH₁₋₃₉ or together with 166 pg ml⁻¹ of ACTH₁₋₃₉.

Previous experiments on the fetal sheep with chronically implanted vascular catheters have shown relatively small changes in corticosteroid concentration for relatively large rises

Table 1 The output of cortisol from adrenal cells of 120–135-d fetal sheep in response to various ACTH concentrations

	0	ACTH concentration (pg ml ⁻¹)			
		40	166	332	1,660
Cortisol produced (ng per 2 h per g of DNA)	0.4 ± 0.16	1.45 ± 0.4	4.35 ± 1.35	5.86 ± 1.64	6.73 ± 1.98
Percentage increase over baseline	—	222%	867%	1,202%	1,396%

The results are means ± s.e.m. of nine experiments.

in ACTH⁷⁻⁹. In contrast to the *in vivo* data, adrenal cells prepared from fetal sheep of 120–135 d gestation respond by causing a marked increase in cortisol output in response to low concentrations of ACTH₁₋₃₉ (Table 1). Note particularly the effect of increasing the concentration from 40 to 330 pg ml⁻¹ and to 1,660 pg ml⁻¹ which represents approximately the basal to the elevated concentration of ACTH₁₋₃₉ in plasma of fetal sheep for experiments carried out *in vivo*⁷⁻⁹. An interpretation of the *in vivo* compared with the *in vitro* data is that *in vivo* something is suppressing the ability of the fetal adrenal to respond to circulating ACTH, although a direct comparison is not easy. This does not appear to be a low PO₂ (unpublished observations). The proportional increase in cortisol output by the fetal adrenal cells between 40 and 330 pg of ACTH per ml,

was of the same order as that found in adrenal cells prepared from the glands of adult guinea pigs, man, rats and sheep¹³⁻¹⁵.

The pituitary of the fetal sheep contains ACTH-like peptides of high molecular weight which are secreted^{12,16} and found in the circulation¹⁷. The proportion of these to ACTH₁₋₃₉ falls in the preparturient period¹⁸. The effects of these peptides on cortisol responses of adrenal cells have shown consistent results in four fetal sheep (Fig. 1). They illustrate that the large peptides (even A, $P < 0.05$) have steroidogenic activity at 1 ng ml⁻¹, but inhibit the response of the ACTH₁₋₃₉ (Fig. 1). It is difficult to exclude the possibility that there is proteolytic breakdown of the large peptides to ACTH₁₋₃₉, as occurs in plasma; were that occurring *in vitro*, it would mask part of the inhibitory action of the three peptides, thus accounting for the impression of the absence of inhibition in some cases.

These three peptides are present in the pituitary of the fetal sheep and are secreted by pituitary cells. The concentration of high-molecular-weight ACTH-like peptides in the plasma (mostly peptides similar to A and B) of fetal sheep at 120–135 d is about 150 pg ml⁻¹ compared with a value of about 50 pg ml⁻¹ for ACTH₁₋₃₉ (ref. 18).

Thus the existence of these peptides in the fetal circulation may explain why the fetal adrenal is apparently less responsive *in vivo* than *in vitro*. Moreover, the change in the ratio of the concentration of these to that of ACTH₁₋₃₉ that occurs late in gestation in the fetal sheep could be involved in the initiation of parturition, possibly by influencing the trophic and steroidogenic effects of ACTH₁₋₃₉ on the adrenal. If these big peptides are suppressing the fetal adrenal of the sheep then they provide an

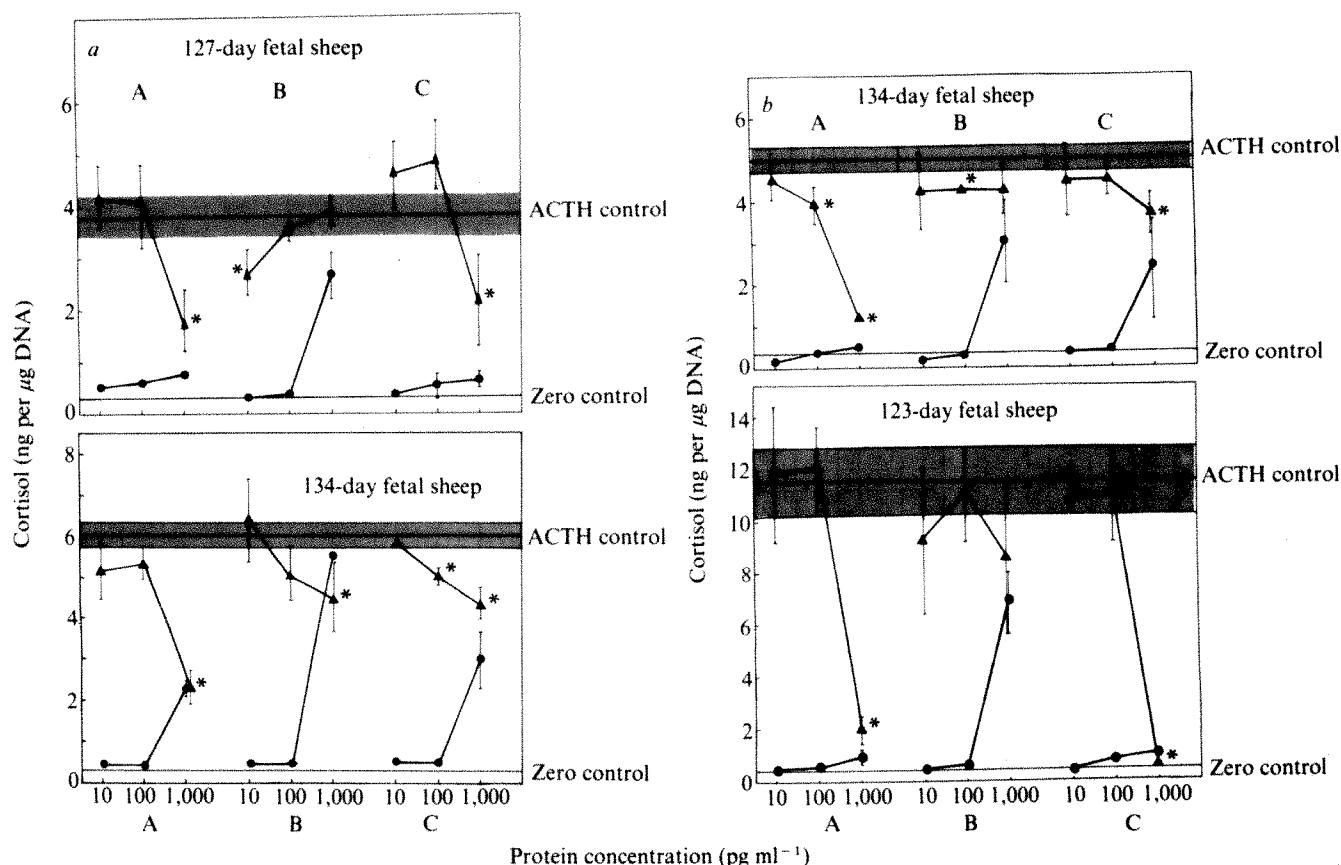


Fig. 1 The response, to ACTH₁₋₃₉ and various ACTH peptides, of adrenal cells prepared from four fetal sheep of 123–134 d gestation. Cells were incubated for 2 h with either 166 pg ml⁻¹ ACTH₁₋₃₉ (ACTH control; the shaded area is equivalent to 2 s.d.), with ACTH₁₋₃₉ plus various concentrations of big peptides (▲), with the big peptide alone (●), or without any addition (zero concentration control). The peptides A, B and C have molecular weights of about 50,000, 30,000 and 20,000, respectively. The values are means ± s.d. for three separate incubations. The rates of cortisol production in the zero controls were 123 d 0.43 ± 0.03, 127 d 0.35 ± 0.07, 134 d 0.39 ± 0.02, 134 d 0.28 ± 0.02 ng of cortisol per 2 h per µg DNA.

* Indicates a significant difference between the response of the cells incubated with ACTH₁₋₃₉ at 100 pg ml⁻¹ as compared with that on incubation with the appropriate peptide plus 100 pg ml⁻¹ of ACTH₁₋₃₉ ($P < 0.05$).

explanation for the paradoxical effect of short-term dexamethasone infusion into the fetal sheep increasing the response to ACTH₁₋₂₄ (ref. 19) as dexamethasone would reduce the secretion of the large peptides as well as that of ACTH₁₋₃₉.

The mechanism by which these large peptides suppress adrenal steroidogenic response to ACTH is not clear. Provided they contain the 15–18 region of the ACTH molecule they should compete effectively for the ACTH receptor and then may block the accessibility of ACTH₁₋₃₉ to the steroidogenic site on the adrenal cell membrane²⁰.

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Autoregulation in rats with transplanted supernumerary kidneys

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Autoregulation of mammalian renal function is implicit in the fact that a few hours after removal of one kidney the activity of the intact nephrons in the other kidney suddenly increases, both in glomerular filtration and tubular reabsorption. There follows a slower but easily measured increase in renal mass—compensatory hypertrophy in each residual nephron. That response to a reduction of nephrons has been well studied¹, but the adaptation of normal renal function to additional nephrons could not be tested until microsurgical techniques made it possible to transplant one or two additional kidneys into normal rats². We report here sequential measurements of the total renal function of rats with three and four kidneys. Our results indicate that autoregulation maintains total renal function at normal levels in spite of a doubling in the number of nephrons and apparent renal mass.

Strictly inbred Lewis rats were used to avoid immunological reaction against the added tissue and to facilitate long-term observations. As before², the donor kidney (or kidneys) was removed *en bloc* with adjacent segments of aorta and vena cava, as well as with the entire ureter and a small patch of bladder.

Recipients were young adult males, whose aorta and vena cava in mid-abdomen was slit anteriorly and joined by sutures of 9.0 nylon to the cuffs of the donor aorta and vena cava. The urine from the added kidney was drained into the recipient's bladder by sewing the patch of donor bladder, which included the ureteral orifices, into a defect cut into the dome of the bladder with 7.0 Dexon. Thus there was no surgical interference with the blood vessels or ureters of the recipient's own kidneys. Unless the donor kidney is spared from ischaemia during transplantation, the resulting acute tubular necrosis of the transplant is particularly severe and the transplanted kidneys atrophy³. We perfused donor kidneys with ice-cold Ringer solution and performed rapid anastomoses. We discarded rats in which the transplants atrophied, as well as those that developed hydro-nephrosis or bladder stones.

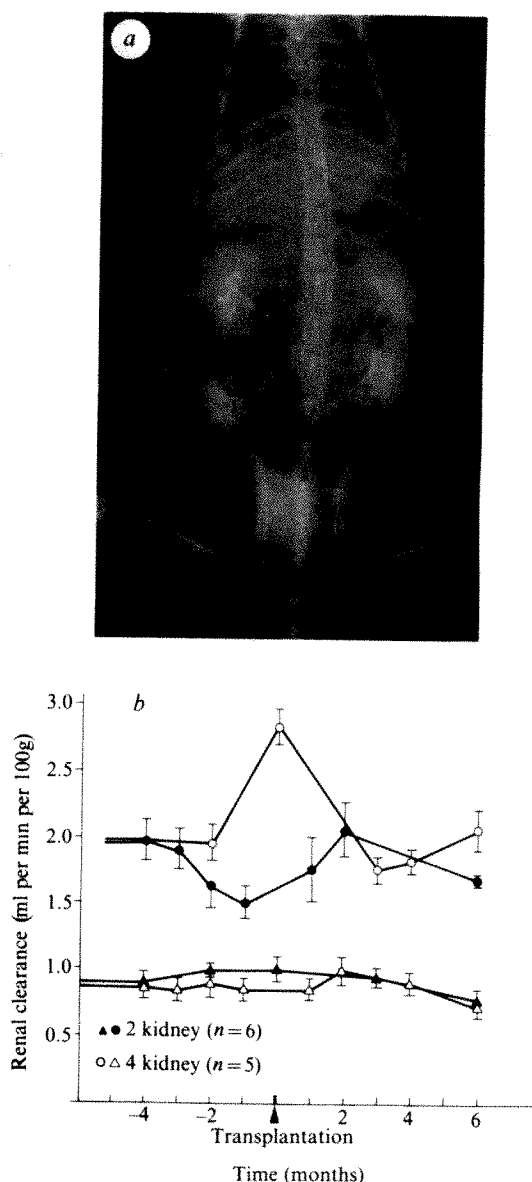


Fig. 1 *a*, Excretory urogram of a four-kidney rat 6 months after transplantation (25 min after injection of iodinated diatrizoate). The added (below) and original kidneys have maintained their size and excrete similar concentrations of contrast. *b*, Sequential renal clearances of ¹²⁵I-iothalamate (GFR, triangles) and ¹³¹I-Hippuran (RPF, circles) relative to body weight in five four-kidney rats (open symbols) and six intact sibling controls (closed symbols). Urograms were prepared with standard diagnostic X-ray equipment on rats anaesthetised with ether, 15–20 min after intravenous injection into the penile vein of 1.5 ml of Renographin 76 (67% diatrizoate, 37% bound iodine, Squibb).

The success of transplantation and the cross-sectional area of the kidneys were demonstrated on excretory urograms during the first week, at 4–6 months and at 1 yr (Fig. 1a). Cross-sectional areas and measurements made with calipers at transplantation and autopsy, showed that in four-kidney rats there was no obvious shrinkage of the original kidneys. We conclude that normal nephron size and aggregate renal mass do not adjust downwards as a consequence of a doubling of the number of nephrons.

Six four-kidney rats were used for long-term studies of glomerular filtration rate (GFR) and plasma renal flow (PRF). We added two kidneys because the doubling of functional mass should override the variability common to the results of clearance studies. A femoral or neck vein was cannulated with polyethylene tubing (PE-50) under ether anaesthesia and the rats were placed in individual metabolic cages (Nuclear Corp.). Saline was infused overnight (2 ml h^{-1} Harvard Instrument infusion pump). The next day, if the urinary output of the awake but restricted rats matched their input, saline was replaced by an isotonic solution of ^{125}I -sodium iothalamate (Glofil, Abbott) and ^{131}I -sodium iodohippurate (^{131}I -Hippuran, NEN) to measure GFR and RPF, respectively. Infusion was continued for another 5 h. Urine produced during the first hour was discarded and then all urine was collected for 4 h: its volume was measured and its radioactivity counted. To obtain blood counts we took blood at the beginning and the end of the collection period by warming the tails and making a cut in each of them. The two isotopes were measured simultaneously in a γ counter (Nuclear Chicago) with appropriate settings. The clearance of both isotopes was calculated as ml min^{-1} and adjusted for body weight. Although simultaneous counting of both isotopes introduced a systematic apparent lowering of the absolute values of RPF, it did not affect the comparison with the controls similarly processed.

GFR and RPF were measured three times in 4 months preoperatively and again at monthly intervals after surgery for 4 more months, and at 6 months for surviving rats. Total GFR and RPF increased with age and with increasing body weight during those 10 months in both groups, but not as a consequence of adding two kidneys. The stability of their GFR and RPF relative to body weight before and after transplantation is shown in Fig. 1b.

We next measured the relative contribution of each kidney towards overall clearance of the isotopes from the blood. We used dimercaptosuccinic acid (DMSA) a mercurial compound taken up selectively by the proximal convoluted tubules in the renal cortex. When bonded to the γ -emitter technetium-99 ($^{99\text{m}}\text{Tc}$ -DMSA), it is used clinically for scintiscanning of functioning renal cortex, usually to compare the uptake of two kidneys in one individual. We examined the uptake of $^{99\text{m}}\text{Tc}$ -DMSA in each kidney of six rats with three-kidneys, 7–9 months after transplantation, and of their intact sibling controls. We injected approximately $1 \mu\text{Ci}$ of $^{99\text{m}}\text{Tc}$ -DMSA (Medi Physics) intravenously: 16 h later anterior and posterior images (Fig. 2a) were recorded by a γ camera with a pinhole collimator (Sigma 400, Ohio Nuclear) and stored on a digital computer (Gamma-11, Digital Equipment). The count rate of the syringe containing the isotope was also stored on the computer before and after injection to determine the activity injected. Renal uptake was expressed for each kidney as a percentage of activity injected, corrected for background. Each value was an average of the uptake measured on the anterior and posterior projections.

Total uptake of DMSA varied from 21 to 30.5% of the injected dose, and in paired controls from 23.7–35%, thus being the same in rats with three kidneys as in a normal rat (Fig. 2b). Uptake by each of the two original kidneys varied from 7.6 to 12.3% of the injected dose, while for the controls the range was 10.3–18.2%. Paired measurements, made on the same day, always showed the uptake of each kidney in the control rat to be greater than that of either of the two original kidneys in the rat with three kidneys. The group difference on analysis by a

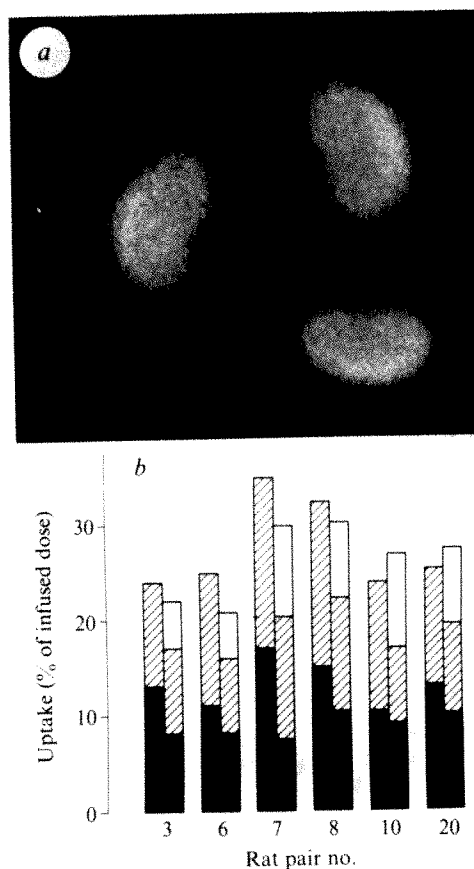


Fig. 2 a, Scintigram of three-kidney rat 16 h after injection of $^{99\text{m}}\text{Tc}$ -DMSA, in posterior view. The right kidney is uppermost and the transplanted third kidney lies transversely below the other two. b, Uptake of DMSA by the six three-kidney rats and sibling pair controls. The total uptakes of the three-kidney rats (right bars) are the same as those of controls (left bars). But the uptakes of the original two kidneys (the right kidney is black and the left is hatched in each bar) are consistently reduced ($P < 0.001$) in the three-kidney rats to accommodate the function of the added third kidney (clear sections of right-hand bars).

binomial probability table⁴ for a sample of 12 with no overlap is highly significant ($P < 0.0002$).

Taken together, our measurements demonstrate that the individual function of intact kidneys was reduced in response to the addition of functional renal tissue. Silber and Crudup tentatively concluded that a third kidney increased GFR by 50% (ref. 5), and an increase was suggested but not proved in our earlier work³. Our findings now conclusively show no increase in rats with three or four kidneys. Kidneys can increase their glomerular filtration and tubular reabsorption work acutely without an increase in size, as noted after uninephrectomy⁶. It now seems that on a day-to-day basis the kidney is capable of a wide range of autoregulation of function, including downward modulation, in response to the excretory needs of the whole body.

A feedback signal seems to enable the aggregate renal glomerular and tubular clearance of rats with two, three or four kidneys to remain a constant linear function of body weight. The signal is probably humoral because the transplanted kidneys responded as well as the intact kidneys. Another hint of a feedback signal comes from the work of Kover and Tost⁷, who connected the circulation of dogs to an isolated two-kidney organ preparation, acutely reducing the urine volume and urine sodium excretion of the original kidneys in proportion to that of the isolated kidneys.

The lack of detectable decrease in kidney size is consistent with the concept that a minimum renal size is achieved during normal growth and is 'obligatory'⁸, not influenced by low functional demand. Thus a baby (4 weeks) rat with a transplanted adult kidney grows up and has normal sized kidneys

(unpublished results of R. A. Dieppa, R.F.G., A.B. and J. McDonald). By contrast, compensatory hypertrophy is reversible to the 'obligatory' baseline size: transplanting a normal kidney into a rat living on a greatly hypertrophied half of one kidney (after removal of one and a half kidneys) reverses the hypertrophy of the half kidney⁹.

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Dopaminergic nerve endings visualised by high-resolution autoradiography in adult rat neostriatum

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Nerve cells containing dopamine (DA), noradrenaline (NA) or serotonin possess powerful physiological mechanisms for the reuptake and storage of their own transmitter. Monoaminergic neurones may therefore be visualised by high-resolution autoradiography¹, provided that tritiated biogenic amines are retained in their sites of uptake and/or storage during suitable preparative histological procedures. Using this method, noradrenergic and serotonergic neuronal cell bodies and axon terminals have been specifically identified and examined in various parts of mammalian central nervous system². However, although the nerve cell bodies of central dopaminergic neurones have been similarly investigated after *in vivo* labelling with either ³H-DA or ³H-NA^{3–7}, the autoradiographic detection of dopaminergic nerve endings has remained problematic. Indeed, in previous studies after cerebroventricular infusion, superfusion or local instillation of ³H-DA or ³H-NA, light and electron microscope autoradiographs of neostriatum, the brain region richest in dopaminergic nerve endings, failed to exhibit the small clusters of silver grains typical of monoamine-labelled axonal varicosities^{8–10}. This suggested tracer displacement during histological processing, so we tested, after intraventricular administration of ³H-DA, a technique of tissue preparation developed in our laboratory for high-resolution autoradiographic visualisation of deoxyglucose-6-phosphate, a diffusible, water-soluble, unbound substrate¹¹. We report here that the rapid successive vascular perfusion of both a glutaraldehyde primary fixative and osmium tetroxide postfixative, which may be followed by dehydration and resin-embedding also carried out by perfusion, maintains a sufficient amount of ³H-DA *in situ* to ensure light and electron microscopic identification of DA axon terminals in rat brain¹².

All experiments were carried out in adult Sprague-Dawley rats (200–400 g), pretreated with a monoamine oxidase inhibitor (β -phenylisopropylhydrazine, 5 mg per kg intraperitoneally, 18 and 2 h earlier) and anaesthetised with sodium pentobarbital. 100–200 μ Ci of 3,4-dihydroxy [*ring*-G-³H]phenylethylamine

hydrochloride (³H-DA, specific activity 6.3 Ci mmol⁻¹, Amersham), diluted in 100–200 μ l of saline containing 1% ascorbic acid, were instilled stereotactically into a lateral cerebral ventricle for 1–2 h. In some cases, non-radioactive NA or serotonin (10⁻³ M) was added to this solution at a molarity 2–10 times higher than that of the tracer. Immediately thereafter, fixation was initiated by high flow-rate aortic-arch perfusion with 600 ml of 3.5% glutaraldehyde in 0.1 M cacodylate buffer (200 ml min⁻¹) and continued without interruption by perfusion with 500 ml of 0.5% osmium tetroxide in 0.2 M cacodylate buffer (same rate). Subsequent dehydration and embedding were also carried out by perfusion, using 3–4 l absolute ethanol and 3 \times 200 ml Epon-propylene oxide mixtures at increasing resin concentrations. The whole brain was then immersed for several hours in pure Epon and polymerised *in toto* at 60 °C¹¹. Samples of the caudate nucleus, ipsilateral to the instilled ventricle, were processed as semi-thin (1 μ m-thick) and thin (silver-gold) sections for light or electron microscope autoradiography, respectively, according to standard dipping techniques¹³. Other brain regions were examined by light microscope autoradiography only.

Three types of autoradiographic reactions were detected in neostriatum after intraventricular administration of ³H-DA. (1) Diffuse labelling, in the form of dispersed silver grains, overlaid all cytological constituents of the paraventricular region, with relative sparing of myelinated bundles. This ubiquitous reaction spread over 150–750 μ m and attenuated gradually with increasing distance from the ependyma. Its intensity was markedly diminished if non-radioactive NA had been added to the ³H-DA solution, indicating a common affinity of both catecholamines for nonspecific and/or artefactual binding sites. (2) Ependymal labelling (not illustrated) consisted of clumps of silver grains seemingly aligned along the ventricular edge in light microscope autoradiographs. In electron microscope autoradiographs, silver grains in this location mostly overlaid intracellular lysosome-like corpuscles predominating in the apical portion of ependymocytes, and plurivesicular or dense granular blebs protruding at the ventricular surface. Such labelling was not found after the addition of either non-radioactive NA or serotonin to the ³H-DA solution, suggesting competition between all three biogenic amines for binding at these ependymal sites. (3) Small and dense aggregates of silver grains, disseminated throughout the neuropil of paraventricular neostriatum, were the main finding of the present study (Fig. 1a). These were typical of labelled axonal varicosities having taken up and retained tritiated biogenic amine in high concentration¹⁴. They were most numerous immediately beneath the ependyma and also densely grouped in deeper patch-like areas extending between some myelinated fibre bundles. Electron microscope autoradiography confirmed that all such accumulations of tracer involved axonal enlargements containing synaptic vesicles. In fact, after a short period of exposure and low-sensitivity development, these were practically the only sites of silver grain localisation (Fig. 1b).

The above experiments give some indication of the signal-to-noise value of the axonal accumulations of ³H-DA. First, these were no longer visible when non-radioactive NA had been added to the ³H-DA solution, even though rat neostriatum receives little if any NA innervation¹⁵. This finding was consistent with the notion of competitive uptake of the two catecholamines in dopaminergic nerve endings^{16–18}. Second, the addition of non-radioactive serotonin to the ³H-DA solution had no apparent effect on either the number or the labelling intensity of the axonal accumulations, as would have been expected from a cross-reactivity of serotonergic nerve endings, abundant in neostriatum. Accordingly, there was no apparent labelling, even after the administration of ³H-DA alone, neither of the supraependymal serotonergic plexus nor of serotonergic axonal varicosities in supra-chiasmatic nucleus, two regions readily accessible to tracer. The latter observations were at variance with previous *in vitro* data indicating that serotonergic fibres have a capacity to take up exogenous DA¹⁹. The apparent discrepancy

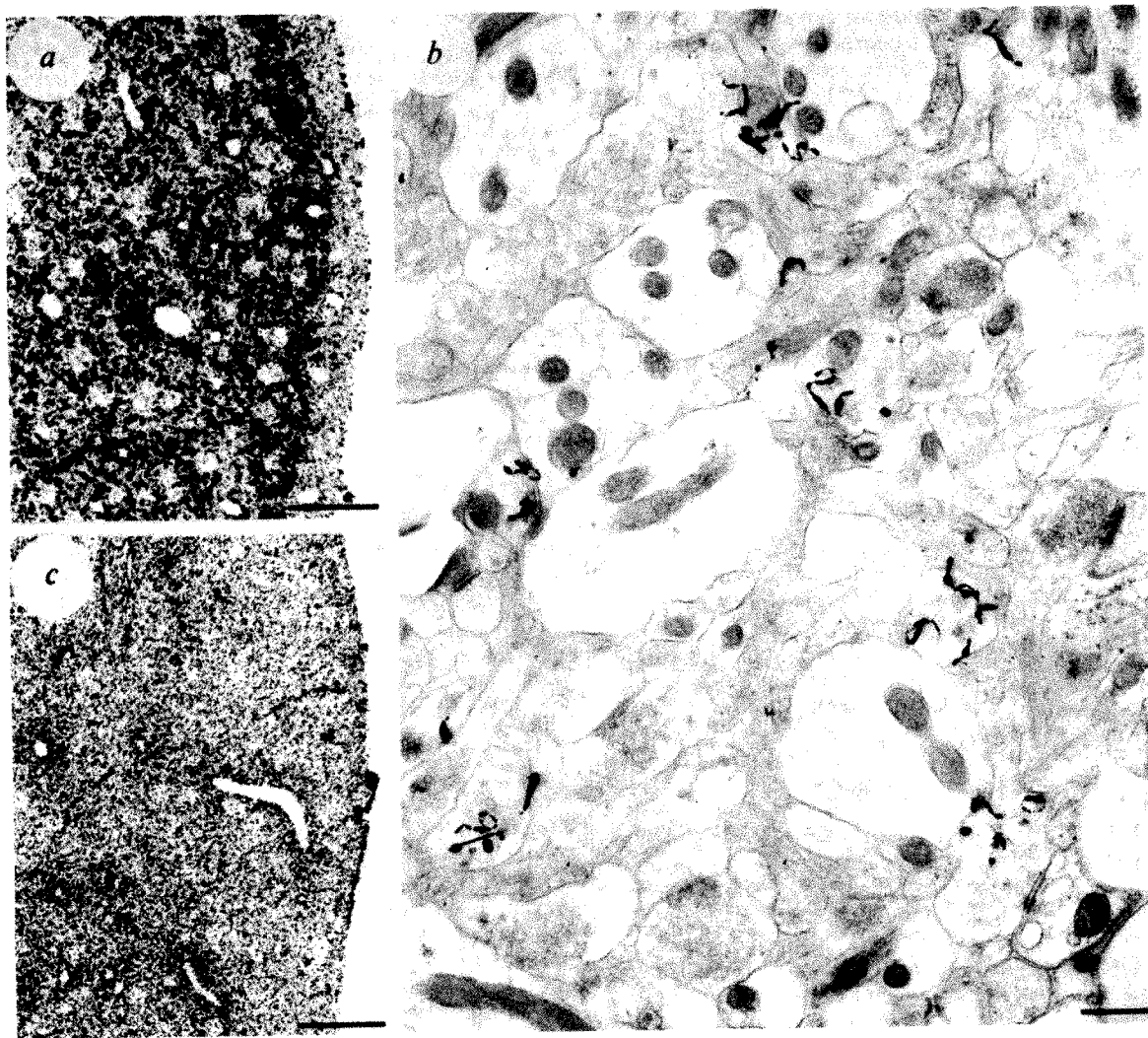


Fig. 1 Light (*a*, *c*) and electron microscope (*b*) autoradiographs of paraventricular neostriatum, after intraventricular instillation of ^3H -DA *in vivo* followed by rapid double perfusion-fixation with glutaraldehyde and osmium tetroxide solutions. *a*, ^3H -DA was administered together with non-radioactive serotonin. Small and dense aggregates of silver grains, disseminated throughout the neuropil, signal the existence of axonal varicosities having taken up and retained the tracer. *b*, All accumulations of silver grains correspond to axonal enlargements containing synaptic vesicles. Some of these varicosities are seen in synaptic junction with dendritic profiles. *c*, ^3H -DA was administered 15 d after destruction of nigrostriatal dopaminergic neurones by 6-hydroxydopamine. The silver grain aggregates are no longer visible, which demonstrates their exclusive dopaminergic axonal origin. *a*, And *c*, D-19 development after 15 and 30 d of exposure, respectively. Scale bars, 50 μm . *b*, Paraphenylenediamine development after 30 d of exposure. Scale bar 0.5 μm .

between results of *in vivo* and *in vitro* experiments could be due to altered properties of severed serotonergic axon terminals incubated *in vitro*, and/or insufficient tissue concentration of ^3H -DA *in vivo* to induce interspecific labelling of serotonergic nerve endings.

Definite proof of the specific identity of the ^3H -DA-accumulating nerve endings in neostriatum was provided by autoradiographic data obtained after 6-hydroxydopamine lesioning²⁰ of the nigrostriatal dopaminergic system. In these rats, the neuronal cell bodies giving rise to the dopaminergic innervation of neostriatum, located in groups A-9 and A-10 of zona compacta and area ventralis tegmenti²¹, were selectively destroyed by local microinjection of the drug (8 μg in 4 μl of saline), 15 d before ^3H -DA administration. In agreement with previous reports, this pretreatment resulted in the conspicuous disappearance of all histofluorescent perikarya in substantia nigra, accompanied by a 90% decrease of endogenous DA content and total loss of histofluorescence in neostriatum²²⁻²⁴. Its consequence in light microscope autoradiographs of neostriatum was the virtual absence of any ^3H -DA

accumulations, confirming their dopaminergic axonal origin (Fig. 1*c*).

It seems likely that the greater diffusion of ^3H -DA than other biogenic amines during standard histological processing reflects some basic difference in the binding of these molecules inside their respective axon terminals²⁵. In fact, by comparison with noradrenergic nerve endings elsewhere in brain, the dopaminergic terminals of neostriatum are known to exhibit less distinct endogenous histofluorescence when visualised with the Falck-Hillarp technique²⁶; they also show a linear fluorescence-concentration relationship, as if some endogenous DA and/or its fluorophore were extracted from 'granules' or even from axoplasm during histochemical processing²⁷. Furthermore, greater amounts of endogenous or exogenous DA than NA are recovered in supernatant when synaptosomes or particulate fractions from regions containing these amines are being prepared by differential centrifugation^{25,28-30}. Such findings have already led to the suggestion that a significant proportion of the biogenic amine within dopaminergic nerve terminals is located extravesicularly, in a weakly bound and readily releasable

pool³¹. This same hypothesis could also explain why the cytochemical demonstration of small dense-core vesicles in neostriatal axon terminals has only been possible after loading with exogenous NA or false transmitter³²⁻³⁵, but has always failed with DA even after tissue incubation in high concentrations of this amine³².

The mechanisms by which histoprocessing by vascular perfusion permits the retention of loosely bound DA in neostriatal dopaminergic nerve endings remain to be elucidated. Preliminary experiments suggest that comparable results may be obtained after successive perfusion of both glutaraldehyde and osmium fixatives and subsequent dehydration and embedding carried out by immersion of tissue slices in the usual manner. It will be of interest to determine whether factors such as the velocity of the double fixation achieved by vascular perfusion, and/or the preservation of unbroken cell membranes throughout glutaraldehyde and osmium fixations, contributes in maintaining ³H-DA *in situ*.

In any event, the present study offered the first opportunity to examine dopaminergic nerve endings of neostriatum in conditions allowing not only their unequivocal identification, but also adequate preservation of their ultrastructural features. Until now, examination of some 100 sectional profiles of dopaminergic axonal varicosities in the paraventricular zone of neostriatum has shown that these are relatively small, averaging 0.5 µm in diameter (0.3–0.9 µm). Most contain round and clear small synaptic vesicles (30–50 nm), usually accompanied by one or more mitochondria; only a few exhibit large dense-core vesicles (80–120 nm). Note also that a fair number of ³H-DA-labelled varicosities may be seen in close apposition to small nerve cell bodies or in symmetrical synaptic junction with dendritic processes (Fig. 1b). Further elucidation of the fine structural features of neostriatal dopaminergic axonal varicosities should help to resolve present controversies regarding the junctional versus non-junctional relationships of these nerve terminals³⁵.

In regions of the brain known to receive a mixed dopaminergic and noradrenergic innervation (for example hypothalamic paraventricular nucleus and median eminence), the distribution of axon terminals detected in light microscope autoradiographs suggests that both types of catecholaminergic nerve endings are simultaneously labelled with ³H-DA after double perfusion of glutaraldehyde and osmium fixatives. Selective cytolytic removal of noradrenergic afferents and/or pharmacological inhibition of their uptake systems, might therefore prove necessary for achieving a specific autoradiographic identification of dopaminergic nerve endings within such parts of the nervous system.

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Role of NK cells in tumour growth and metastasis in beige mice

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Although natural killer (NK) cells are thought to give the host a spontaneous resistance against tumours and have been postulated to act *in vivo* as surveillance cells¹, definitive data in support of these hypotheses has not been obtained. Recently the beige (bg) mouse, a morphological homologue of the human Chediak-Higashi (CH) syndrome²⁻⁴, was shown to be deficient in NK activity⁵. Specifically, spleen cells of bg mice were demonstrated to be incapable of *in vitro* natural cytotoxicity against tumour cells⁵. We report here that a tumour line, modified to be sensitive to NK cytotoxicity by *in vitro* culture, demonstrated *in vivo* an increased growth rate, faster induction time and an increased metastatic capability in bg compared to control mice. This was not found with a tumour line insensitive to NK activity (without *in vitro* culture). *In vivo* activation of NK cells in bg and control mice resulted in a decrease in tumour growth rate and metastatic frequency. These results demonstrate that NK cells have an important function in the host's control of tumour growth and metastasis.

NK cells seem to be pre-T cells that do not require thymic maturation. This could account for their presence in athymic nude mice⁶. The level of NK cell activity is genetically controlled⁷, age-dependent⁸ and may be augmented by numerous agents, including infection with lymphocytic choriomeningitis virus (LCMV)⁹. Induced NK-cell activity peaks 3 d after injection of LCMV and decreases to normal levels by day 8 (ref. 10). Interferon levels directly parallel the rise and decrease of NK activity¹¹ suggesting that the increased NK-cell activity in LCMV infected mice is mediated by the viral induction of interferon¹⁰. The sensitivity of tumour cell lines to NK-cell activity varies directly with the length of time they have been cultured *in vitro*, and *in vivo* passaged cell lines are often insensitive to NK cytotoxicity⁸. At least 3 weeks of *in vitro* culture are required for a tumour line to develop an increased sensitivity to NK cytotoxicity¹².

Growth parameters and metastatic abilities of the malignant melanoma tumour B16 were studied in syngeneic normal (+/bg) and bg (bg/bg) C57BL/6 mice. B16 tumours which were passaged *in vivo* and cultured *in vitro* only a short time were insensitive to NK lysis (Table 1A). However, increased *in vitro* culture time resulted in the acquisition of a sensitivity to NK lysis

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by the tumour cells (Table 1A). Table 1 also demonstrates that spleen cells from normal mice exhibit NK activity but that spleen cells from *bg* mice do not exhibit unstimulated NK activity, confirming a previous report⁵. LCMV induction of NK activity markedly enhanced cytotoxicity against B16 cells cultured *in vitro* for 32 d as well as against B16 cells cultured *in vitro* for only one day that were refractory to unstimulated NK activity (Table 1A).

The injection of LCMV into 6–8-week-old mice increased NK activity in both normal and *bg* mice. In normal mice NK activity peaked on day 3 and returned to normal by day 6 following LCMV injection. The *bg* mice expressed NK activity 24 h after LCMV injection. This activity peaked on day 5, and decreased more slowly than in normal mice, a finding in agreement with that of Kiessling¹².

Tumours insensitive to NK lysis required the same time to achieve 100% tumour induction and grew to essentially the same mean tumour volumes in normal and *bg* mice (Table 2). However, tumour cells sensitive to NK lysis had longer tumour induction times, slower growth rates (data not shown) and produced smaller tumours in normal compared to *bg* mice (Table 2). The parameters of tumour growth for both the NK-insensitive and -sensitive tumour lines were reduced when injected into LCMV-infected normal or *bg* mice (Table 2). Spontaneous metastasis was assessed following subcutaneous tumour growth in a pinna, followed by resection of the ear on day 21, and necropsy of mice at the onset of morbidity¹³. Approximately the same number of metastases were found in normal and *bg* mice injected with NK-insensitive tumour cells

Table 1 Sensitivity of B16 cultures to NK lysis and activation of NK cells in beige (*bg/bg*) and normal (+/*bg*) mice

A Effect of various intervals of *in vitro* culture on the sensitivity of B16 cells to NK cell lysis from the spleens of *bg* and normal mice

Length of B16 culture <i>in vitro</i> (d)	Per cent cytotoxicity											
	Effector cells <i>bg/bg</i> (Effector to target ratio)						Effector cells +/ <i>bg</i> (Effector to target ratio)					
	200:1	100:1	50:1	200:1	100:1	50:1	200:1	100:1	50:1	200:1	100:1	50:1
1	0.0	0.0	0.0	5.6	0.0	0.0	52	52	38			
1	0.0	0.0	0.0	0.0	0.0	0.0	40	44	32			
1	0.0	0.0	0.0	4.6	0.0	0.0	50	44	28			
32	2.0	0.0	0.0	14.0	8.8	2.0	64	64	42			
32	3.3	0.0	0.0	14.0	8.6	5.0	68	56	34			
32	2.1	0.0	0.0	20.0	10.0	6.6	66	56	42			

B Time of appearance of activated NK cells in the spleens of *bg* and normal mice following LCMV injection

Days postinfection	Per cent cytotoxicity			
	Effector cells +/ <i>bg</i>		Effector cells <i>bg/bg</i>	
	B ₁	B ₂	B ₁	B ₂
0 Control	8	10	4	3
1	28	34	6	12
2	28	34	16	20
3	40	42	28	36
4	12	14	32	42
5	14	16	38	46
6	8	8	26	28

A, Cultured target cells were labelled with ⁵¹Cr (2×10^7 cells were incubated with 200 μ Ci ⁵¹Cr for 40 min at 37 °C), washed, allowed to attach to the wells of a microtest II dish (5,000 cells per well) for 4 h, and washed again. Complete medium (200 μ l) containing effector cells from the spleens of: (1) *bg*; (2) normal; or (3) normal mice infected 3 d previously with $1-2 \times 10^4$ plaque-forming units (PFU) of LCMV were then added. Following 6 h incubation at 37 °C 100- μ l aliquots of the supernatant were removed and counted in a γ counter. Spontaneous release was obtained from wells incubated without effector cells and was always less than 10% of total release obtained with 2% SDS (450 cpm/6,000 cpm total release). NK activity is expressed as the per cent cytotoxicity = (test c.p.m. – spontaneous c.p.m.)/(total c.p.m. – spontaneous c.p.m.).

B, NK lysis activity was assayed as in experiment A, using B16 target cells which had been cultured *in vitro* for 32 d. Mice that were 6–8 weeks of age were injected with $1-2 \times 10^4$ PFU of LCMV on consecutive days and assayed on the same day using an effector: target ratio of 100:1.

Table 2 Role of NK cells in modifying subcutaneous growth and metastasis of B16 tumour cells

Time required to reach 100% tumour induction, or the percentage of animals with tumours 21 days following tumour injection

Mouse genotype	LCMV	B16 cells cultured for:	
		5 days	32 days
<i>bg/bg</i>	–	13 days	14 days
+/ <i>bg</i>	–	14 days	45%
<i>bg/bg</i>	+	16 days	17 days
+/ <i>bg</i>	+	80%	80%

Mean tumour size \pm s.e.m. 21 days following tumour cell injection (mm³)

Mouse genotype	LCMV	B16 cells cultured for:	
		5 days	32 days
<i>bg/bg</i>	–	105 \pm 26	93 \pm 22
+/ <i>bg</i>	–	175 \pm 57	4.1 \pm 1.2
<i>bg/bg</i>	+	81 \pm 36	42 \pm 19
+/ <i>bg</i>	+	25 \pm 15	5.4 \pm 1.4

Incidence of pulmonary metastases (mean number of metastases \pm s.e.m. per mouse)

Mouse genotype	LCMV	B16 cells cultured for:	
		5 days	32 days
<i>bg/bg</i>	–	5.4 \pm 0.95	7.1 \pm 0.88
+/ <i>bg</i>	–	6.1 \pm 0.63	0 \pm 0
<i>bg/bg</i>	+	0 \pm 0*	0 \pm 0*
+/ <i>bg</i>	+	0 \pm 0*	0 \pm 0

The mice received 10^5 cells in a 0.05-ml aliquot subcutaneously in one ear. Mice with LCMV had received $1-2 \times 10^4$ PFU 2 days previously. The tumour size was measured in three dimensions after 21 days of growth and the volume calculated¹⁷. The tumour bearing ear was amputated at this time. 15 days later the mice were necropsied and the number of spontaneous metastases counted. $n = 10$ mice.

* Several of these mice had extensive peripheral lymph node tumour growth.

Table 3 Role of host NK activity and tumour sensitivity to NK lysis in modifying the incidence of metastases following intravenous injection of B16 tumour cells

A	Mouse genotype	LCMV	Mean no. lung metastases \pm s.e.m. per mouse	
			B16 tumour cells cultured for:	
			5 days	32 days
	<i>bg/bg</i>	–	6.7 \pm 0.88	8.4 \pm 2.71
	+/ <i>bg</i>	–	9.2 \pm 1.48	0 \pm 0
	<i>bg/bg</i>	+	3.1 \pm 0.5	4.2 \pm 0.73*
	+/ <i>bg</i>	+	3.3 \pm 0.31	0 \pm 0
B			2 days	40 days
	<i>bg/bg</i>	–	17.4 \pm 2.27	16.8 \pm 2.78
	+/ <i>bg</i>	–	11.0 \pm 1.45	0 \pm 0

Mice received 0.10 ml of a single cell suspension of B16 cells (experiment A, 20,000 cells; experiment B, 50,000 cells). These cells had been cultured for 5 or 32 days (experiment A), and 2 or 40 days (experiment B). The mice injected with LCMV received $1-2 \times 10^4$ PFU of LCMV 2 days before tumour cell injection. After 15 days the mice were necropsied and the number of pulmonary metastases counted using a dissecting microscope. $n = 10$ mice.

* These mice had five extra pulmonary metastases, three ovarian and two renal.

(Table 2). However, the incidence of spontaneous metastasis was markedly reduced with NK lysis sensitive tumour cells injected into normal mice compared to *bg* mice. The metastatic incidence was greatly reduced in mice with LCMV-activated NK cells bearing NK-insensitive tumours in normal or *bg* mice and NK-sensitive tumours in *bg* mice.

The metastatic abilities of NK-sensitive and -insensitive B16 cells were also studied using the lung colony assay (Table 3). No difference was found in the experimental metastatic ability of NK-insensitive tumour cells in normal or *bg* mice (Table 3). However, NK-sensitive tumour cells were metastatic using the lung colony assay in *bg* mice but not in normal mice (Table 3). Metastatic ability seemed to correlate with the tumour cells' sensitivity to NK lysis and with the level of NK activity. This conclusion was further substantiated using mice of both genotypes infected with LCMV. These mice had fewer lung colony foci following injection with either NK lysis sensitive or insensitive tumour cells compared to mice without activated NK cells, except for NK-sensitive cells injected into normal mice where they were already non-metastatic.

A role for NK cells in metastasis was initially suggested by the observation that transplanted tumours rarely metastasise in nude mice¹⁴ which have high levels of NK activity¹⁵. Also there is a direct correlation between the level of NK activity in different mouse strains and the short-term survival of tumour cells labelled with ¹²⁵I-iodoxuridine using a lung colony assay¹⁶. An NK-sensitive tumour injected into *bg* mice (deficient in normal NK activity) is induced faster, grows more rapidly and has more metastases than the same tumour in normally NK-constituted mice. These differences were not present with a tumour insensitive to NK cells. Normal and *bg* mice with

LCMV-activated NK cells, however, exhibited a reduced growth rate and reduced metastatic incidence of both NK lysis sensitive and insensitive tumour cells. These results suggest an important role for NK cells in the host's control of malignant disease.

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Low natural *in vivo* resistance to syngeneic leukaemias in natural killer-deficient mice

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Natural killer (NK) cells, which occur at high levels in normal non-immune individuals of several species, have the capacity to lyse certain tumour cells *in vitro*, and have been proposed as a first level of defence against tumour growth *in vivo*¹⁻³. Evidence for this comes mainly from the murine system, where certain F₁ hybrid or T-cell deficient mice with high NK activity resist growth of transplanted tumours better than do normal syngeneic controls with lower NK activity⁴⁻⁶. Recent studies of the beige mutation on the C57BL mouse strain background, previously proposed as an animal homologue of the human Chediak-Higashi syndrome^{7,8}, have revealed a new model for analysing anti-tumour effector mechanisms in the intact syngeneic host^{9,10}. The recessive beige gene (*bg*⁺), which affects melanosome and granulocyte lysosome functions^{11,12}, also causes a profound (but not total) depression of NK activity whereas other anti-tumour effector mechanism mediated by T cells and macrophages remain relatively intact¹³. It was important, therefore, to test the tumour susceptibility of beige mice as a first direct test of the hypothesis that NK cells are involved in surveillance against neoplasia. In partial confirmation of this hypothesis, we report here that failure of small threshold doses of one virally and one chemically induced transplantable leukaemia to grow out in syngeneic mice partly depends on a rapidly acting host defence mechanism. This mechanism is deficient in *bg/bg* mice, which develop palpable, progressively growing tumours faster and at a higher frequency than do phenotypically normal *+/bg* littermates.

The benzpyrene-induced EL-4 and the Rad-LV-induced P-52-127-166 leukaemias of C57BL/6 origin, are both highly resisted by certain C57BL F₁ hybrids that have high NK activity *in vitro*^{14,15}. Also, low-dose inocula of the *in vivo* maintained ascites lines of the two leukaemias behaved similarly; subcutaneous (s.c.) inoculation in a transplantation test yielded higher tumour take incidence in *bg/bg* mice than in heterozygous littermates (Fig. 1). The differences were most striking in

the early phase of the observation period, with a majority of the total tumour takes in *bg/bg* registered within 2 weeks (68% with EL-4, 69% with P-52-127-166) whereas only a small part of the tumours in *+/bg* mice were palpable at this time (31% with EL-4, 14% with P-52-127-166). Thus, in addition to the increased incidence of takes in mutant mice, the progressively growing tumours appeared with a shorter latency than in controls. This was also reflected in the larger mean tumour diameter by 2 and 3 weeks and the earlier deaths among tumour-bearing *bg/bg* than *+/bg* mice (Fig. 1 legend).

The outcome of a long-term transplantation test can be influenced by a variety of host factors. However, most of the tumours in *bg/bg* mice had appeared after the short period of 2 weeks, after which the tumour incidence curves of *bg/bg* and *+/bg* became virtually parallel. This indicated that the defect in natural resistance of beige mice involved early events after tumour inoculation. Further support for this was obtained in a test system where the short-term survival of intravenously (i.v.) injected ¹²⁵I-iododeoxyridine (IUdR) labelled leukaemia cells is monitored, previously proposed as a most appropriate *in vivo* assay for natural rejection mechanisms^{16,17}, including NK cells^{18,19}. The heterozygous control mice eliminated P-52-127-166 ascites cells more efficiently than did beige mice, measured either as total, pulmonary or splenic radioactivity retained 18 h after i.v. injection (Fig. 2), although in the spleen, differences were already detectable after 4 h (Fig. 2 legend). The results suggest that *bg/bg* mice may lack an important mechanism for rapidly eliminating tumour cells, for example, as blood-borne metastases in the lungs and spleen.

Our findings in *in vivo* tests correlate well with the *in vitro* measured splenic NK activity against both tumours tested. EL-4 or P-52-127-166 cells had a low, but significant, sensitivity to *+/bg* spleen cell cytotoxicity (5-10% specific lysis) whereas *bg/bg* spleen consistently gave values below 3% specific lysis (Fig. 3).

Augmentation of the low NK levels against EL-4 and P-52-127-166 occurred in mice receiving the low tumour cell dose used in the transplantation tests (Fig. 3). The NK activities of *+/bg* as well as *bg/bg* mice were increased, although relative differences between the groups were maintained. In both tumour-inoculated and control groups, *bg/bg* mice gave values corresponding to a ninefold dilution of *+/bg* splenocytes in terms of lytic units. As has been shown with larger doses of tumour cells²⁰, the augmented cytotoxicity observed here was mediated by non-adherent cells (Fig. 3) which were relatively resistant to treatment with anti-Thy 1.2 plus complement, and declined in activity after day 3 to values comparable to those in non-inoculated control mice (unpublished observation). Such augmented NK activity, induced during the transplantation

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tests, may account for *in vivo* resistance against tumour cells which show very low susceptibility to *in vitro* lysis by NK cells from normal mice.

Several different phenotypic manifestations of the beige mutation may be partly responsible for the low natural resistance against transplanted tumours in *bg/bg* mice observed here. However, our results from the two *in vivo* test systems suggest that early events are of major importance for the final outcome of tumour growth; this would argue against adaptive mechanisms requiring proliferative expansion of specific clones, as in a cytotoxic T-cell response. Furthermore, the early more efficient *in vivo* elimination of leukaemia cells in *+/bg* mice was paralleled by a greater cell-mediated cytotoxicity *in vitro* against the same tumours which was independent of adherent cells. This makes the involvement of monocytes or macrophages, proposed as responsible for natural surveillance in other systems^{21,22}, less likely. Finally, *bg/bg* mice generate highly efficient T-killer cells *in vivo* after injection of P815 tumour cells, and anti-tumour effects mediated by macrophages are also normal¹³. Although we cannot exclude the possibility that mechanisms which are

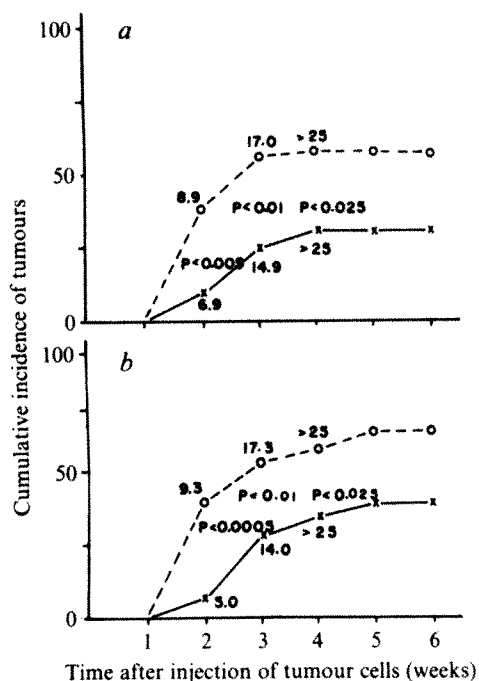


Fig. 1 Cumulative tumour take incidences in *+bg* and *bg/bg* mice. EL-4 or P-52-127-166 cells were collected from the ascitic fluid of C57BL/6 mice (in which the tumours were propagated *in vivo* in our animal colony) and washed once in balanced salt solution (BSS). EL-4 (5×10^3) or P-52-127-166 (10^3) cells were then injected s.c. in the back of *+bg* or *bg/bg* littermates, obtained by crossing C57BL/6 *+bg* and C57BL/6 *bg/bg* breeders (originally purchased from Jax Laboratories and maintained by continuous brother-sister mating according to a backcross-intercross scheme in our animal colony). The challenging cell doses were chosen from pretitration experiments so that tumours would grow progressively only in a fraction (30–40%) of syngeneic *+bg* mice. By this procedure, the inoculum would be large enough to be able to establish a growing tumour mass, but still so small that it would allow detection of any impairment of natural resistance in *bg/bg* mice by an increased incidence of tumours. Curves and *P* values (χ^2 -test) compare the tumour take incidence in *+bg* (x) and *bg/bg* (o) at weekly intervals, with figures at each time point referring to mean tumour diameter (out of three perpendicular measurements) in mm. a), EL-4 (four experiments with a total of 52 *+bg* and 38 *bg/bg* mice tested): out of all *+bg* mice that developed progressively growing tumours, 6% had died by week 3 and 65% by week 4; the corresponding figures for *bg/bg* mice were 22% by week 3 and 95% by week 4. b), P-52-127-166 (6 experiments with a total of 70 *+bg* and 56 *bg/bg* mice tested): out of all *+bg* mice that developed progressively growing tumours, 0 had died by week 3, 21% by week 4 and 69% by week 5; the corresponding figures for *bg/bg* mice were 6%, 57% and 89%, respectively. No new palpable tumours appeared after 5 weeks (mice were kept for 8–12 weeks after tumour injection). Preliminary experiments with 10-fold higher tumour cell doses have yielded 80–90% takes in beige as well as control animals, although latency and survival data show faster tumour growth in *bg/bg* mice.

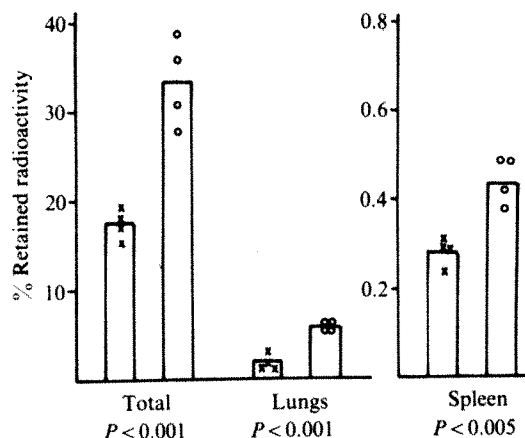


Fig. 2 *In vivo* elimination of $^{125}\text{IUdR}$ -labelled leukaemia cells in *+bg* and *bg/bg* mice. P-52-127-166 ascites cells were labelled by intraperitoneal injection of $50 \mu\text{Ci } ^{125}\text{I}$ -labelled 5-iodo-2'-deoxyuridine ($>5 \text{ Ci per mg}$, Radiochemical Centre) 3 days after passage of 10^7 cells. After 6 h, cells were collected by peritoneal washes, counted, washed four times in BSS and injected in a lateral tail vein of *+bg* and *bg/bg* mice (1×10^6 cells per mouse). Groups of four or five animals were killed after 4 and 18 h, and the spleens and lungs were removed. Whole body and organ counting were carried out in an Intertechnique γ spectrometer. Symbols represent total (whole body + spleen + lungs), splenic and pulmonary retained radioactivity (calculated as percentage of total injected) in individual *+bg* (x) and *bg/bg* (o) mice after 18 h. Vertical columns represent arithmetic means, and *P* values refer to statistical analysis by a Student's *t*-test. Four hours after injection, the corresponding values were as follows (mean and range): *bg/bg*, 83 (71–89); *+bg*, 82 (77–80); lungs: *bg/bg*, 61 (57–69); *+bg*, 54 (49–60); *P* < 0.1; spleen: *bg/bg*, 0.52 (0.47–0.56); *+bg*, 0.41 (0.33–0.52), *P* < 0.05.

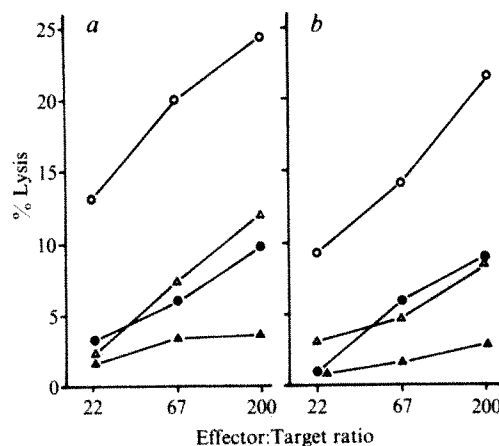


Fig. 3 Splenic NK activity of normal or tumour-inoculated *+bg* and *bg/bg* mice. Three days after s.c. inoculation of BSS or 10^3 P-52-127-166 cells, mice were killed and spleen cell suspensions prepared in complete RPMI with 10% fetal calf serum. Cells from three pooled spleens were incubated in a nylon wool column²⁶ and thereafter used as effectors in a standard 8-h ^{51}Cr release assay (as previously described) against EL-4 (a) or P-52-127-166 (b) cells. Values represent the mean of triplicate samples (triplicate variation < 5% of mean value). Spontaneous release was 11.2% for EL-4 and 15.1% for P-52-127-166. Unfractionated control cells were in all cases slightly less efficient than nylon wool-passed cells as cytotoxic effectors (not shown). ●, *+bg* (BSS); ○, *+bg* (10^3 P52-127-166); ▲, *bg/bg* (BSS); △, *bg/bg* (10^3 P52-127-166).

unaffected by the beige mutation contribute to natural resistance, our results indicate that NK cells have a major role in the cell-mediated *in vivo* defence against transplanted leukaemias observed in syngeneic phenotypically normal (*+bg*) mice. This has important implications for the evaluation of the influence of NK cells on primary tumour formation and growth in the normal autochthonous host. If the high NK-cell activity of nude mice is the explanation for their low or 'normal' incidence of tumours and their ability to resist growth of certain murine as well as human transplanted tumours, it is clear that a double mutant combining the beige and the nude defects would be a useful tool

for future research. Such a defect is not lethal, and we have recently been able to produce double mutant mice by a series of crosses and intercrosses between C57BL/6 *bg/bg* and C57BL/6+/*nu* mice. These C57BL/6 *bg/bg*, *nu/nu* mice are now being characterised immunologically.

In conclusion, the experimental model provided by the beige mutation provides strong additional support for the notion that NK cells may be involved in anti-tumour surveillance *in vivo*. Conclusive evidence should come from further studies of primary induced or spontaneous tumours in beige mice. In this context, it is interesting that humans bearing the Chediak-Higashi gene have an even greater impairment (500-fold) in NK

function²³ and a corresponding increase in the incidence of a spontaneous lymphoproliferative disorder which is thought to be malignant²⁴.

During these studies, we became aware of a similar study in progress by Talmadge *et al.*²⁵ (*Nature*, this issue), showing faster growth and increased metastasis of *in vitro* cultured syngeneic melanoma cells in *bg/bg* mice.

We thank Dr Mona Hansson for helpful discussions, and Ms Maj-Lis Solberg and Ms Margareta Hagelin for technical assistance. This investigation was supported in part by grants 1R01 CA 26782-01 and 1R01 CA 14054-07 from the NCI, DHEW, and by grants from the Swedish Cancer Society.

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Retinoids specifically enhance the number of epidermal growth factor receptors

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Retinoids elicit many biological and biochemical responses from cells *in vitro*¹⁻⁵. One widely used criterion for the responsiveness of cells to retinoids is inhibition of growth; retinoids reduce the saturation density and/or growth rate of many normal and tumorigenic cell lines^{2,6,7}. Propagation of eukaryotic cells has been demonstrated to be dependent on the presence of macromolecular growth factors such as epidermal growth factor (EGF)⁸⁻¹⁰, which can stimulate proliferation of epithelial and fibroblastic cell lines. We now describe the effect of retinoids on the binding of EGF to its receptor. Retinoic acid enhances binding of ¹²⁵I-labelled EGF to various fibroblastic and epidermal cell lines. It has no marked effect on the affinity of this growth factor for its receptor, but increases the number of EGF receptor sites. Retinoic acid has little effect on the binding of concanavalin A (Con A) and insulin, indicating the specific nature of the action of retinoids on cell-surface glycoproteins. Treatment of cells with the phorbol ester 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) and retinoic acid shows poor antagonism between these compounds on EGF binding. It has been previously shown that retinoids induce or stimulate differentiation of embryonal carcinoma cells¹¹⁻¹³. EGF binding can be used as a marker to monitor differentiation of these cells.

When sparse cultures of 3T6 cells are treated with retinoic acid the growth rate and saturation density are reduced, cells become more adhesive and have less tendency to overlap⁶. Figure 1 shows the effect of various concentrations of retinoic acid and its phenyl analogue on the binding of ¹²⁵I-labelled mouse EGF to 3T6 cells. Retinoic acid markedly increases binding of ¹²⁵I-EGF to its receptor, whereas the phenyl analogue does not affect this binding. Maximum binding, five- to sevenfold above control levels, occurs at a concentration of

3×10^{-6} M retinoic acid. The effect of retinoic acid on EGF binding is reversible; 3 days after the removal of retinoic acid, levels of EGF binding are reduced to those of control cells (not shown).

Because for certain cell lines, EGF binding is strongly dependent on cell density^{15,16}, the retinoic acid-induced enhancement of EGF binding might be related to the reduction of saturation density caused by this compound. However, we found no dependency of EGF binding on cell density in either retinoic acid-treated 3T6 cells ($1-6 \times 10^4$ cells cm^{-2}) or control cells ($1-12 \times 10^4$ cells cm^{-2}).

To determine the structural specificity of retinoids for stimulating EGF binding, we studied the effect of several analogues on the binding of this growth factor to 3T6 cells (Table 1). Retinoic acid and its *cis* analogue are the most potent derivatives tested, whereas the phenyl, pyrimidyl, methyl ether and ethylamide analogues are inactive. Retinol and retinyl acetate also increase EGF binding, although less effectively than retinoic acid. The specificity of the various analogues for stimulating EGF binding parallels that with which they increase adhesiveness and reduce saturation density of 3T6 cells² and induce differentiation of embryonal carcinoma cells¹³. Furthermore, except for retinol and retinyl acetate, which do not bind to the retinoic acid-binding protein, the effect of all analogues on EGF binding correlates exactly with their capacity to compete with retinoic acid for the binding site of this binding protein^{2,13,17}. We have found that retinyl acetate is rapidly converted to retinol and that 3T6 cells do not contain detectable amounts of retinol-binding protein (unpublished results). The activity of both retinol and retinyl acetate, therefore, cannot be due to the involvement of this binding protein but may be explained by the conversion of retinol to a metabolite that is able to bind effectively to the retinoic acid-binding protein.

To investigate whether the enhancement of EGF binding induced by retinoids is due to either an increase in unoccupied receptors or increased affinity, the effect of EGF concentration was determined. As shown in Fig. 2, retinoic acid has little effect on the affinity of ¹²⁵I-labelled mouse EGF for its receptors. Scatchard plot analyses indicate dissociation constants of 4.1×10^{-10} M and 4.5×10^{-10} M for retinoic acid-treated and control cells, respectively. However, the number of unoccupied EGF receptor sites is markedly increased from 2.87×10^4 receptors per cell for control cells to 1.51×10^5 receptors per cell after retinoic acid treatment.

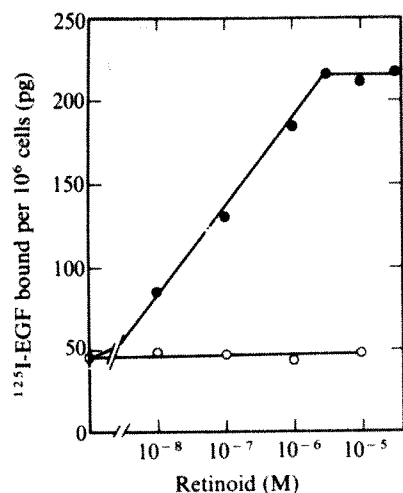


Fig. 1 Effects of retinoic acid at various concentrations on the binding of ^{125}I -labelled mouse EGF to 3T6 cells. Cells were grown in DMEM containing 10% calf serum for 3 days in the presence of either retinoic acid dissolved in ethanol or ethanol alone (0.15% v/v final concentration). After trypsinisation cells were subcultured in 35-mm cluster dishes ($1-2 \times 10^5$ cells per well). After 48 h incubation, binding of EGF was determined in duplicate as described by Todaro *et al.*^{16,18}. The medium was aspirated and the cells washed twice with 2 ml binding buffer (DMEM containing 1 mg ml^{-1} bovine serum albumin and 50 mM N,N -bis-(2-hydroxyethyl)-2-aminoethanesulphonic acid adjusted to pH 6.8). In the binding assays 1 ml binding buffer containing 0.825 ng of ^{125}I -labelled mouse EGF (Collaborative Research, 1.4×10^5 d.p.m.) was added to each well. After 60 min at 37°C unbound ^{125}I -EGF was removed, cells were washed four times with 2 ml binding buffer and solubilised in 0.75 ml lysing buffer (0.1 M Tris-HCl, pH 7.4, containing 0.5% SDS and 1 mM EDTA) and the wells washed twice with 0.5 ml lysing buffer. Radioactivity was counted in a Packard γ -counter. Nonspecific binding was determined in the presence of $10 \mu\text{g}$ unlabelled mouse EGF (Collaborative Research) and consisted of 100–300 d.p.m. per 10^6 cells. Cell number was determined in a Coulter counter; in all cases binding was determined at equal cell density ($\sim 5 \times 10^5$ cells per well). ●, Retinoic acid; ○, phenyl analogue of retinoic acid.

To test the generality of its effect on EGF binding, we studied the action of retinoic acid on several other cell lines and two other ligands, Con A and insulin (Table 2). Retinoic acid increases the binding of EGF in several other mouse fibroblast cell lines, including two clones of 3T12, two of 3T3 and one 3T3 derivative obtained by transformation with the chemical carcinogen dimethylbenzanthracene. Another 3T3 derivative transformed by benzo(a)pyrene shows very low levels of EGF binding which are not increased by treatment with retinoic acid. Also, melanoma S91M3 cells show very low binding of EGF, whereas treatment with retinoic acid enhances this binding. Retinoic acid stimulates severalfold the binding of EGF to two mouse epidermal cell lines, JB-6 and its tumorigenic derivative T62 obtained after selection by treatment with TPA¹⁸ but has little effect on EGF binding to human epidermoid carcinoma cells A431 and to Syrian hamster kidney cells BHK-21. Treatment of 3T3 cells with retinoic acid inhibits cell growth, enhances cell-to-cell substratum adhesiveness, increases production of glycosaminoglycans and stimulates EGF binding, whereas none of these characteristics is affected in its virus-transformed derivative 3T3SV. This difference can be attributed, respectively, to the presence and absence of retinoic acid-binding protein in these two cell lines².

Retinoic acid treatment has little effect on Con A and insulin binding (Table 2). It increases Con A binding to 3T6 and 3T12 C1A cells by 41% and 29%, respectively, but this binding is virtually unchanged in 3T3 and 3T3SV cells. Retinoic acid does not affect binding of insulin to any of these cell lines. These results indicate a specific nature of the action of retinoids on cell-surface glycoproteins.

Table 1 The effect of retinoids on the binding of ^{125}I -labelled mouse EGF to 3T6 cells

Basic chemical structure	R	^{125}I -EGF binding (pg per 10^6 cells)	Relative binding
	CH_2OH^*	230.4	2.71
	$\text{CH}_2\text{OCOCH}_3$	257.6	3.03
	COOH^\dagger	483.2	5.68
	CH_2OCH_3	97.0	1.14
	COOH	324.3	3.81
	CONHC_2H_5	91.2	1.07
	COOH	478.2	5.63
	COOH	83.2	0.98
	COOH	356.0	4.18
	COOH	71.6	0.84

Cells were grown for 5 days in the presence of 10^{-5} M of the indicated retinoid. The binding of ^{125}I -EGF was determined as described in Fig. 1 legend; 1.25 ng ^{125}I -EGF per ml (counts 1.2×10^5 d.p.m.) was added. Control cells bound 85.0 pg per 10^6 cells. The relative binding is defined as binding of ^{125}I -EGF to retinoid-treated cells/binding to control cells.

* , Retinol; † , retinoic acid.

Several reports have shown antagonism between retinoic acid and the tumour promoters phorbol esters. Retinoic acid inhibits induction of ornithine decarboxylase by phorbol esters¹⁹ and prevents growth in soft agar induced by sarcoma growth factor and phorbol esters^{20,21}. Phorbol esters reduce EGF binding by decreasing its affinity for its receptor¹⁴. When TPA is added to 3T6 or 3T12 C1A cells, EGF binding is reduced within 5 h, whereas EGF binding to retinoic acid-treated cells increases gradually with time, becoming optimal after 3 days of treatment (Table 3). Cells incubated simultaneously for 5 h with TPA and retinoic acid show no antagonism between these compounds on

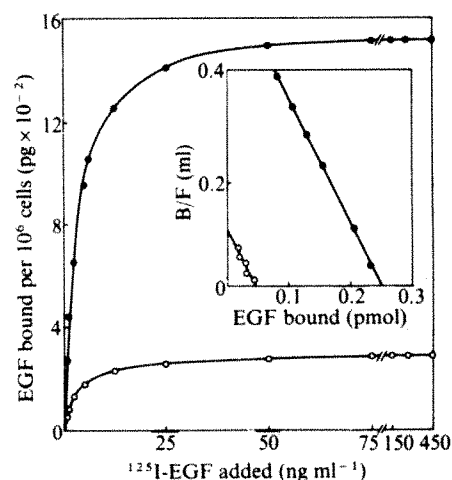


Fig. 2 Effect of EGF concentration on binding of ^{125}I -labelled mouse EGF to retinoic acid-treated and control 3T6 cells. Cells were treated with 10^{-5} M retinoic acid for 5 days. The binding assays were carried out as described in Fig. 1 legend. Data in the main figure were used for Scatchard plot analyses (inset). ○, Control; ●, retinoic acid treated cells; B/F, bound/free EGF.

Table 2 Effect of retinoic acid on the binding of EGF, Con A and insulin to various cell lines

Cell type	Designation	Binding of ^{125}I -ligand (10^3 d.p.m. per 10^6 cells)					
		Control	EGF RA-treated	Control	Con A RA-treated	Control	Insulin RA-treated
Mouse fibroblast	3T6	3.82	25.98 (6.8)	48.40	68.24 (1.41)	1.06	0.89 (0.84)
Mouse fibroblast	3T12CLA	2.18	16.54 (7.6)	32.36	41.83 (1.29)	0.61	0.60 (0.98)
Mouse fibroblast	BALB/3T3	4.59	15.14 (3.3)	32.66	35.10 (1.07)	0.54	0.53 (0.98)
Mouse fibroblast	BALB/3T3SV	4.92	5.93 (1.2)	22.10	21.70 (0.98)	0.57	0.57 (1.0)
Mouse fibroblast	3T12	4.16	9.66 (2.3)				
Mouse fibroblast	BALB/3T3A31-1-1*	1.74	6.20 (3.56)				
Mouse fibroblast	BALB/3T3A31-1-BP-2*	0.14	0.16 (1.14)				
Mouse fibroblast	BALB/3T3A31-1-DMBA-1*	2.68	11.42 (4.26)				
Mouse epidermal	JB-6†	3.70	9.36 (2.53)				
	T62†	1.74	5.90 (3.4)				
Mouse melanoma	Cloudman S-91 M3	0.03	0.20 (6.7)				
Syrian hamster kidney	BHK-21	3.81	3.04 (0.8)				
Human epidermoid carcinoma	A431‡	51.12	49.44 (0.97)				

The binding assays were carried out in duplicate as described in Fig. 1 legend. All cells were grown in DMEM containing 10% calf serum except the melanoma S91M3 cells, which were grown in Dulbecco's modified Eagle's medium (DMEM) containing 15% horse serum and 2.5% fetal calf serum. All cells were treated for 5 days with 10^{-5} M retinoic acid (RA) or with ethanol in the control and were at equal density (5×10^5 cells per 35-mm dish) at the time of the binding assay. The specificities of the ligands used were: ^{125}I -labelled mouse EGF 0.825 ng ml^{-1} (9.5×10^4 d.p.m. per ng) was added, with $10 \mu\text{g}$ of unlabelled EGF to correct for nonspecific binding; ^{125}I -labelled Con A (NEN) 25 ng ml^{-1} (9.8×10^5 d.p.m.) and 15 mg ml^{-1} of α -methyl-D-mannoside to correct for nonspecific binding; ^{125}I -labelled insulin (Sigma) 2.5 ng ml^{-1} (2.7×10^5 d.p.m.) and $20 \mu\text{g ml}^{-1}$ unlabelled insulin to correct for nonspecific binding. The relative binding (binding of ^{125}I -labelled ligand to retinoic acid-treated cells/binding to control cells) is given in parentheses.

*Obtained from Dr T. Kakunaga; †obtained from Dr. N. Colburn; ‡obtained from Dr G. J. Todaro.

Table 3 Comparison of the action of retinoic acid and TPA on the binding of EGF to 3T6 and 3T12 C1A cells

Cell line	Treatment	^{125}I -EGF binding (pg per 10^6 cells)	Relative binding
3T6 (grown in the presence of 0.15% ethanol)	Control	87.0	1.0
	+TPA, 5 h	4.8	0.06
3T6 (grown in the presence of 10^{-5} M RA for 5 d)	Control	443.9	5.10
	+TPA, 5 h	77.7	0.89
3T12 CLA (grown in the presence of 0.15% ethanol)	Control	34.1	1.0
	+TPA, 5h	5.4	0.16
	+RA, 5 h	48.4	1.42
	+RA, 24 h	93.5	2.74
	+RA, 48 h	131.7	3.86
3T12 CLA (grown in the presence of 10^{-5} M RA for 5 d)	+TPA, +RA, 5 h	5.6	0.16
	Control	175.6	5.15
	+TPA, 5 h	86.5	2.54

Cells were treated with 100 ng ml^{-1} TPA and with 10^{-5} M retinoic acid (RA) at the times indicated. Controls received identical amounts of dimethyl sulphoxide or ethanol. EGF binding was assayed as described in Fig. 1 legend, EGF concentration was 1.25 ng ml^{-1} (1.1×10^5 d.p.m. per ng).

the binding of EGF. Cells grown for 5 days in the presence of retinoic acid also exhibit a reduced binding on TPA treatment, although this decrease is relatively smaller than in control cells. Thus, retinoic acid poorly antagonises the effect of TPA on EGF binding. Similar results were obtained for two epidermal cell lines, JB-6 and T62 (L.D. Dion and A.M.J., unpublished results). The above results seem to indicate an essential difference between the mode of action of retinoids and phorbol esters on EGF receptors: the slow action of retinoids on the cell surface seems to involve metabolism and/or macromolecular syntheses whereas the rapid action of phorbol esters may reflect conformational changes induced by alterations in the fluid state of the membrane²².

We have shown recently that retinoic acid stimulates differentiation of embryonal carcinoma cells PCC4 aza 1R (refs 12, 13). During 24–48 h of treatment with retinoic acid these

cells differentiate irreversibly into a cell type with a fibroblastic morphology. The differentiated cells can be distinguished from the original embryonal carcinoma cells by a variety of cell-surface markers¹². The PCC4 aza 1R cells exhibit very low levels of EGF binding whereas the differentiated derivative PCC4 D shows much higher levels of binding (Table 4). Therefore, EGF binding can be used as a marker to monitor differentiation of PCC4 aza 1R cells. After 24 h of treatment with retinoic acid, EGF binding increases concomitantly (not shown) with the appearance of cells with a fibroblastic morphology and the production of plasminogen activator which was used previously to monitor differentiation of PCC4 aza 1R cells¹². Similar results were recently reported for other embryonal carcinoma cell lines²³. The phenyl analogue of retinoic acid, which is unable to stimulate differentiation of embryonal carcinoma cells, has no stimulatory effect on EGF binding.

Thus, retinoic acid treatment of several cell lines enhances the binding of mouse EGF to its receptors, not by changing its affinity but by increasing the number of available receptor sites. Stimulation of EGF binding may result from an increase either in total receptor sites or in the number of unoccupied sites alone. Certain cell lines are known to produce endogenous growth factors²⁰, some of which may bind to the EGF receptor;

Table 4 Increase in EGF binding during differentiation of embryonal carcinoma cells PCC4 aza 1R induced by retinoic acid

Cell line	Treatment	^{125}I -EGF binding (pg per 10^6 cells)
PCC4 aza 1R	Control	1.2
	24 h RA	1.4
	48 h RA	20.3
	72 h RA	58.9
	96 h RA	112.7
	96 h ethanol	1.5
PCC4 D	96 h phenyl analogue	1.3
	Control	67.1
	96 h RA (10^{-5} M)	143.4

Cells were grown in DMEM containing 10% fetal calf serum. Differentiation was induced by the addition of 10^{-6} M RA or ethanol in the control. EGF binding was determined at the time intervals indicated, 1 ng EGF per ml (1.2×10^5 d.p.m.) was used. PCC4 D is the differentiated derivative obtained from PCC4 aza 1R after cloning¹¹.

retinoids may suppress the production of these factors leading to an increase in the number of unoccupied receptors and decreased cell growth. Alternatively, retinoids may increase the synthesis of EGF receptors. The implications of the increase in EGF receptor for cell growth remain to be established. Preliminary results show that EGF can enhance mitotic activity in serum-starved retinoic acid-treated cells, indicating that retinoids do not totally block the EGF response²⁶.

The action of retinoids on EGF binding provides us with a tool for studying the involvement of retinoid metabolites, like mannosylretinyl phosphate^{1,7}, in glycosylation of glycoproteins and a fast screening test for retinoids potentially active in inhibiting carcinogenesis^{24,25}.

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Vitamin C preferential toxicity for malignant melanoma cells

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Vitamin C has been suggested^{1,2} and disputed³ as an anti-cancer agent. For cells in culture, no preferential effect against any type of cancer has yet been demonstrated. Our aim here is to show that vitamin C is selectively toxic to at least one type of malignant cell—a melanoma—at concentrations that might be attained in humans. Copper ions react with ascorbate and generate free radicals in solution⁴. Ascorbate when combined with copper rapidly reduces the viscosity of DNA solutions and has exhibited some carcinostatic effects on transplanted sarcoma 180 tumours in mice⁵. We reasoned that the elevated copper concentration in melanoma^{6,7} could result in a more selective toxicity for ascorbate.

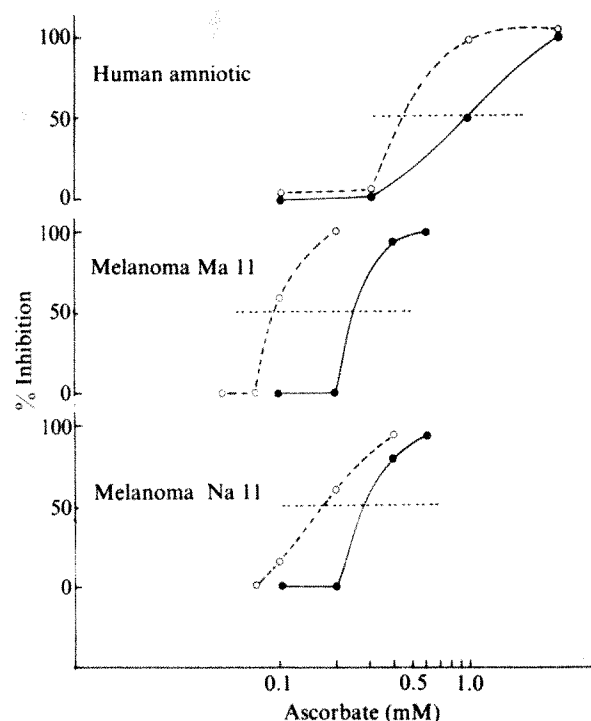


Fig. 1 A typical experiment on two melanoma and one normal cell line determining the reduction in the number of colonies by ascorbate only (●); or ascorbate + 5 μ M copper (○). Colony formation, which directly measures reproductive death, was carried out as previously described⁸. Briefly, about 200-500 single cells were inoculated into culture boxes and the number of colonies containing more than 50 cells was counted 8-14 days later. Colony-forming efficiency of parallel controls ranged from 25 to 60% for the different cell lines studied with this technique.

Inhibition was obtained from:

$$\frac{\text{No. of untreated control colonies} - \text{No. of treated colonies}}{\text{No. of untreated control colonies}}$$

Each point represents the average of a triplicate determination.

Cells (see Table 1) were grown in McCoy's medium supplemented with 15% fetal calf serum and antibiotics. Cytotoxic effects were determined using three independent techniques with parallel simultaneous runs on a melanoma and a non-melanotic cell culture (see Fig. 1). Drugs were added to cells 16-24 h after inoculation with single cell suspensions. Sodium ascorbate (Sigma) was added to cells from a water stock solution within 2 h of preparation.

Table 1 summarises the results obtained on 13 cell lines in four different laboratories. The concentration of ascorbate which resulted in a 50% decrease in colony formation, cell number or viability was between 0.2 and 0.5 mM (a mean of 0.3) for all the melanotic cell cultures studied. Cloned cell lines with many trypsinisations were as sensitive as cells recently put into culture. No obvious difference relating to the degree of pigmentation was noted. Ascorbate was added to Na11 cells which were suspended in agar⁸ 30 min after trypsinisation, and in another instance 30 h after inoculating into a Falcon box, yet the differences in the ID₅₀ were not significant. Plots of percentage inhibition with the three measuring techniques used not only gave similar ID₅₀ concentrations, but also exhibited a very sharp increase in inhibition at about this value (Fig. 1).

The non-melanoma cells tested (Table 1) were less sensitive to ascorbate and these other cell types exhibited a much larger range in their ID₅₀ values. Only mouse embryo fibroblast cultures showed an ascorbate sensitivity approaching that of melanoma cells.

A catalytic concentration of copper (5 μ M or ~ 20 less than the Cu²⁺ ID₅₀) increased ascorbate toxicity for melanoma two- to fivefold (Table 1). Cytotoxicity to other cells increased only 0 to 1.5 times.

Table 1 Effect of ascorbate on the growth of melanoma and other cells

	Cells			ID ₅₀ * (mM)			
	Pigmentation	Doubling time (h)	No. of passage	Measuring technique	Ascorbate	Ascorbate + 5 μ M Cu ²⁺	Cu ²⁺
Melanoma (human)							
FO1 ^a	+	39	100	t.b. ²	0.2	0.05	0.2
Bel ^a	+	30	105	g.i. ¹	0.4	—	—
Val ^a	±	30	20	g.i. ¹	0.4	—	—
Na11 ^b	++	20	160	c.f. ³	0.3	0.1	0.2
				c.f.a. ¹	0.15	—	—
				g.i. ³	0.3	0.07	0.2
Ma11 ^b	+	22	80	c.f. ³	0.25	0.09	0.1
DOR ^c	0	48	100	g.i. ¹	0.4	—	0.25
Mouse B16	+	48	3	c.f. ²	0.5	0.05	0.01
				Average	0.3	0.07	0.15
Non-melanoma							
Normal human amniotic cells ^e		24	60	c.f. ²	1.0	0.6	0.1
				g.i. ³	1.0	0.6	0.2
Human fibroblasts (F1) ^d		24	3	g.i. ¹	0.9	0.9	0.4
Mouse embryo fibroblasts ^f		20	30	c.f. ¹	0.4	0.4	0.2
				g.i. ¹	0.7	0.7	0.2
Transformed							
Chinese hamster ovary		12	>100	t.b. ²	2.0	1.5	0.5
Chinese hamster V79 ^b		16	>100	c.f. ¹	0.7	0.5	0.3
HeLa (human)		24	>100	t.b. ²	1.0	0.8	0.5
				Average	1.0	0.7	0.3

g.i., Growth inhibition measurements were carried out by inoculating 10⁵ cells into 25-cm² boxes (Falcon). Drug exposures from 2 to 5 days with one to four ascorbate additions were studied. Results shown were obtained by addition of ascorbate 1 day after inoculation and by trypsinising and counting cells in a haemocytometer on day 5. Per cent inhibition was calculated from the equation in Fig. 1 using the number of cells counted.

c.f., Colony formation as described in Fig. 1.

c.f.a., colony formation in agar was carried out as by Salmon *et al.*⁸

t.b., Trypan blue exclusion (0.2%) and cell morphology were determined 5 days after one drug addition to about 1,000 cells in Falcon microwell plates. Viability determinations were done each in triplicate.

* Average value calculated from curves like those in Fig. 1.

^{1,2,3}, The number of independent experiments, each performed in duplicate or triplicate.

a, Cells from Dr B. Giovannella; b, see ref. 9 for further details; c, cells from Dr N. Saal; d, cells put into culture in September 1979 from normal adult skin; e, cells from Flow Laboratories; f, C₃H cells from Dr R. H. Bassin.

The results can be described as follows: melanoma cells were 2–10-fold and 5–20-fold, respectively, more sensitive to ascorbate, and to ascorbate plus 5 μ M Cu²⁺ than for amelanotic cells. Referring to the typical inhibition curves of Fig. 1, it can be seen that in 0.6 mM ascorbate the ability of melanoma cells to form colonies is 10 to 20 times less than for normal human amniotic cells. In 1 mM ascorbate no melanoma colonies were observed, while ~300 colonies were counted in controls; yet, at this concentration amniotic cell colony formation was inhibited by only 50%. The ratio of relative inhibition is then ~100-fold here.

With the exception of melanoma, no obvious difference between transformed (malignant) cell lines and normal cells was observed (Table 1). Copper ions alone exhibited some preferential toxicity to melanoma cells. On the other hand, the sensitivity to Cu²⁺ varied greatly from one cell line to another (Table 1), and non-transformed human amniotic cells were killed by lower concentrations of copper than many pigment cells.

The inhibitory effect of copper as sulphate, gluconate and acetate salts was studied. Although the pH of the stock 0.1 M solution added varied from 3.5 with copper sulphate to 5.3 with copper acetate, no difference in the Cu²⁺ ID₅₀ for melanoma cells was observed. Ceruloplasmin, the usual serum transport protein for copper, was not toxic to either melanoma or amniotic cells in the range 10–100 μ g ml⁻¹. However, in a preliminary study, both 7 \times 10⁻⁵ M ascorbate and 30 μ g ml⁻¹ of ceruloplasmin caused a 50% inhibition in colony formation.

Note that the relative growth inhibition of vitamin C for melanoma compared to normal cells at 5 \times 10⁻⁴ M (Table 1) is similar to that reported for vitamin A at 10⁻⁵ M (ref. 10). However, vitamin C is about 1,000 times less toxic than vitamin A.

The only previous report of ascorbate toxicity on melanoma cells was part of a study of the killing effect of dopa and other melanin precursors¹¹. Our results confirm the finding¹¹ that ascorbate reduces the toxic effect of dopa. The pertinent result mentioned in ref. 11 was that 1 mM ascorbate reduced thymidine incorporation in Cloudman S-91 cells by 33% after a

16-h exposure. We obtained a similar inhibition with a 16-h exposure. In our experiments we have found that cytotoxic effects at 2–4 \times 10⁻⁴ M ID₅₀ concentrations of ascorbate become evident only after about 48 h. For non-melanoma cells, our ID₅₀ of 1–2 mM for human amniotic, HeLa, and CHO cultures compares well with values in the literature (Table 2). Vitamin C and copper are required for melanin biosynthesis, and are preferentially incorporated by melanoma tissues^{6,7,16}, yet, at elevated concentrations, both of these agents inhibit dopa oxidase activity^{17,18}. This is one possible mechanism for the specific inhibition of melanoma cell proliferation.

Nonspecific ascorbate toxicity has been attributed to the formation of H₂O₂ during its auto-oxidation^{14,19}, but at pH < 7.6 ascorbate is no longer auto-oxidisable except in the presence of metallic catalysts such as copper¹³. Ascorbate has little or no antimicrobial activity in the absence of metal ions⁴. A cogent example from the early literature is that 6 \times 10⁻⁵ M ascorbate plus 5 μ M Cu²⁺ completely inactivated polio virus after a 4-h contact⁴, while in the presence of EDTA, 6 \times 10⁻⁴ M ascorbate had no effect. Thus it seems reasonable to suggest that the selective toxicity of ascorbate for melanoma is partly due to preferential incorporation of copper. That catalytic concentrations of Cu²⁺ greatly increase the preferential toxicity of ascorbate for melanoma cells, suggests that an intracellular mechanism is involved here.

A likely target for ascorbate killing is DNA. Mutagenic properties of ascorbate and copper have been demonstrated on

Table 2 Ascorbate inhibition

Cell types	Approximate ID ₅₀ (mM)	Refs
Rat plasmacytoma	1.5*	12
Mouse neuroblastoma	1.5*	13
Mouse glioma	2*	13
Ehrlich ascites	10†	14
Chinese hamster V 79	4†	15

* An exposure to ascorbate for more than 36 h was used as in Table 1.

† An exposure of less than 4 h was used.

bacteria and bacteriophage^{21,22} and more recently on eukaryotic cells²³. Vitamin C and copper rapidly eliminate the replication and virulence of bacteria (that is, *Bacillus pestis* of bubonic plague), but do not alter neither morphology antigenicity, or most biochemical reactions over a much longer period of time²¹.

Ascorbate rarely acts as a coenzyme or vitamin but generally modifies the redox potential of the cell. Vitamin C can thereby appreciably potentiate or deactivate many antitumour drugs¹³.

The results presented here indicate that vitamin C may directly inhibit the growth of proliferating cells, and this might explain some of the reported carcinostatic effects². Such an inhibition would depend on the extent to which a given cell incorporates ascorbate, and the sensitivity of the cell to its

cytotoxic effects. Melanoma cells have been found to preferentially incorporate vitamin C (ref. 16), and our *in vitro* studies show that vitamin C is more toxic to melanotic cells than to any others studied so far.

Saturation levels of ascorbate in tissues range from 1 to 2 mM (ref. 24). Such concentrations in our experiments inhibit malignant melanoma cells 20 to 500 times more than the other cell studied. Moreover, as 10^{-5} M free copper concentrations are not toxic in man, the synergistic effect of copper on ascorbate can also be exploited. *In vivo* experiments on mice with melanoma are under way, and clinical studies are planned.

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Vasopressin increases water permeability by inducing pores

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Vasopressin is thought to increase the water permeability of the luminal membrane of distal urinary epithelia either by enhancing the solubility or diffusion of water in the lipid phase¹ or by inducing aqueous pores². Measurement of the permeability to water and small solutes in different experimental conditions should allow the distinction between these two modes of water transport^{1–9}, but because of technical difficulties (for instance the effect of unstirred layers^{3–6}) or conceptual problems (in the case of activation energy measurements^{7–9}) the interpretation of many of these methods is in question. We present here a new approach to this problem using proton conductance of the membrane as a probe for the presence of aqueous channels. Protons travel in free solution by jumping from one water molecule to another¹⁰. If vasopressin induces aqueous pores, it should also increase proton permeability, as protons will be able to jump from a water molecule in the bulk solution to another in the pore. However, if vasopressin changes water permeability by increasing membrane fluidity the proton conductance should not increase as the dielectric constant of even very fluid lipids is low, leading to extremely low solubility of ions in the lipid¹¹. We found that vasopressin increased the proton permeability of the luminal membrane of the toad urinary bladder and conclude that this hormone increases water permeability by inducing aqueous pores rather than by increasing lipid fluidity. Proton conductance may be of use in determining the existence and properties of other aqueous channels.

Vasopressin increased the proton disappearance from an acid mucosal medium bathing Dominican toad bladders, an epithelium which does not actively transport protons (Fig. 1). In 22 experiments vasopressin increased the proton permeability (G_H) by $7.24 \times 10^{-4} \text{ cm s}^{-1}$, a 300% increase.

To investigate the site of the vasopressin-induced proton conductance we measured the intracellular pH using the distribution of the weak acid, dimethyl-oxazolidine-dione

(DMO)¹². Paired bladders treated with vasopressin were placed in a solution containing ^{14}C -DMO and ^3H -inulin at pH 7.4 for 1 h. The mucosal solution of one member of each pair was replaced by a medium of pH 5.0 containing the same concentration of ^3H -inulin and an amount of ^{14}C -DMO calculated to give the same concentration of the free-acid DMO (~4% of the original DMO concentration). After 15 min, the

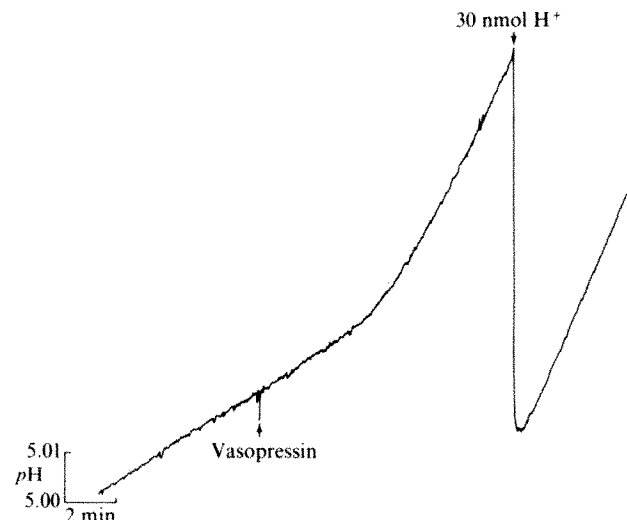


Fig. 1 Effect of vasopressin on H^+ movement across toad bladder. Bladders were placed in Ussing chambers, in short-circuit conditions, using a bicarbonate-free low buffer medium and rates of proton disappearance from the mucosal medium were measured by titration. The figure shows one experiment. Addition of vasopressin to the serosal medium markedly increases the rate of proton disappearance within a few minutes. When the pH reaches 5.1 a measured quantity of acid is added to the luminal medium serving to calibrate proton disappearance and to return the pH to 5. To demonstrate that the proton flux induced by vasopressin results from flow across the epithelium rather than from buffering at the surface, we showed in six experiments that the short circuit current in the presence of amiloride (which abolishes the sodium current) is not different from the simultaneously measured mucosal proton disappearance (0.42 compared with $0.36 \text{ nmol cm}^{-2} \text{ min}^{-1}$). Further, the mucosal proton disappearance and serosal proton appearance in the same bladders were equal (0.34 and $0.33 \text{ nmol cm}^{-2} \text{ min}^{-1}$, $n = 3$).

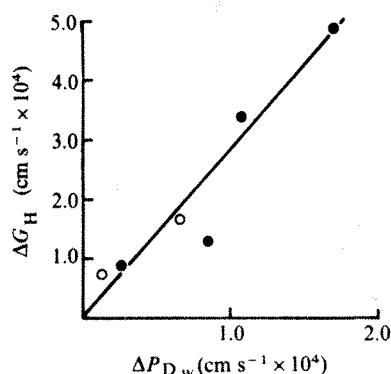


Fig. 2 Plot of the simultaneously measured changes in proton permeability (G_H) and $^3\text{H}_2\text{O}$ permeability (P_D) induced by vasopressin. The proton permeability was measured as the ratio of the flux to the concentration difference.

intracellular pH of scraped epithelial cells from the control bladder was 6.7 while the pH of those exposed to the low mucosal pH was 6.12 ($\Delta\text{pH} = 0.55 \pm 0.12$, $n = 6$). This suggests that the vasopressin-induced proton permeability occurs at the mucosal border of the cell. Further evidence for this was obtained from measurement of the effect of amiloride on H^+ fluxes in the presence of vasopressin. Amiloride increases the negative potential of the toad bladder epithelial cell and hence should increase the electrical gradient for proton movement into cells¹³ while having no effect on the paracellular conductance. In 10 experiments in vasopressin-treated toad bladders, addition of amiloride to the luminal medium increased the proton flux by $0.06 \pm 0.03 \text{ nmol cm}^{-2} \text{ min}^{-1}$ ($P < 0.05$). These results demonstrate that vasopressin increases the proton permeability of the luminal border of toad bladder cells, rather than the permeability of the paracellular pathway.

Simultaneous measurement of G_H and tritiated water permeability (P_D) showed that the increase in G_H correlated with the increase in P_D , $r = 0.947$, $n = 6$ (Fig. 2). Since vasopressin increases the urea and sodium permeability as well as water permeability, we investigated whether protons move through urea or sodium channels. Methohexital, an anaesthetic that inhibits vasopressin-induced water permeability but has little effect on urea and sodium permeabilities¹⁴, reduced the vasopressin-induced proton permeability from $5.80 \times 10^{-4} \text{ cm s}^{-1}$ to $1.44 \times 10^{-4} \text{ cm s}^{-1}$ ($P < 0.01$, $n = 9$). Two results suggest that the protons are not crossing urea channels. We found no correlation between ^{14}C -urea and proton permeability measured in 10 bladders. Furthermore, addition of phloretin, an agent which inhibits urea transport without affecting water permeability¹⁵, abolished the vasopressin-induced urea permeability (control $9.92 \times 10^{-4} \text{ cm s}^{-1}$, 0.1 mM phloretin $0.26 \times 10^{-4} \text{ cm s}^{-1}$, $P < 0.01$, $n = 6$ paired hemibladders) but had no significant effect on the proton permeability measured in the same bladders (control $2.60 \times 10^{-4} \text{ cm s}^{-1}$, phloretin $1.86 \times 10^{-4} \text{ cm s}^{-1}$). It is not likely that protons cross sodium channels as the effect of vasopressin on sodium transport was transient while its effect on proton permeability was not; 30 min after addition of vasopressin the proton flux was 561% above the initial rate while sodium transport was only 25% above the initial value ($n = 14$). Also, as shown above, amiloride abolished sodium transport but increased the proton flux in the presence of vasopressin.

Since protons, being small ions, cannot pass through even very fluid lipid membranes, the above results show that vasopressin must have induced aqueous channels which conduct protons. A rough estimate of the conductance of these channels can be obtained by comparing their proton to water permeability ratio to that of gramicidin A channels. The proton conductance of a single gramicidin A channel at pH 5.0 is 0.015 pS (ref. 16) and its P_D $1.8 \times 10^{-15} \text{ cm}^3 \text{ s}^{-1}$ (ref. 17), giving a ratio of 7.8 S s cm^{-3} . The vasopressin induced proton conductance was $3.22 \mu\text{S cm}^{-2}$ and the P_D increased by $0.78 \times 10^{-4} \text{ cm s}^{-1}$, giving a ratio of

0.04 S s cm^{-3} . The vasopressin-induced channels are at least 200 times less conductive than gramicidin A. Whether this is due to their native ion selectivity or to a very low density of water or to other factors remains to be determined.

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A novel α -globin gene arrangement in man

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The human genome has two linked α -globin genes on chromosome 16. Deletion of one or more of them, as occurs in α -thalassaemia, leads to a reduced output of α -globin mRNA in proportion to the number of α -globin genes lost¹. In some racial groups deletion of one of the pair of α -globin genes may result from unequal crossing over between the genes on homologous chromosomes^{2,3} by a mechanism resembling that postulated for the formation of the $\delta\beta$ fusion genes of the Lepore haemoglobins⁴. By analogy, the opposite chromosome in this cross-over should have three α -globin genes just as the 'anti-Lepore' chromosome has three non- α -chain genes. We describe here a Welsh family in which three members have five α -globin genes—three on one chromosome and two on the other. The additional α gene results in an increased α mRNA output and it may therefore produce the phenotype of mild β -thalassaemia.

DNA was obtained from a 61-yr-old male (E.J.) and his sons (D.J., R.J. and C.J.). Two of the sons (D.J. and C.J.) were subsequently shown to have the same α -globin gene arrangement as their father whereas R.J. had a normal α -gene arrangement. Digestion of normal DNA with the endonucleases *Bam*HI and *Eco*RI, or a combination of the two, generates a single fragment containing both α -globin genes^{2,5,6}. When DNA from E.J. was digested with these enzymes two fragments were obtained, one of normal size and the other consistently 3.5–4.0 kilobases larger (Fig. 1A). This result suggested that E.J. has one chromosome which yields a normal restriction fragment and another which produces a larger fragment with an insertion of approximately 3.5–4.0 kilobases of DNA between the *Eco*RI and *Bam*HI restriction sites which surround the two α -globin genes.

Digestion of normal DNA with *Bgl*II or a combination of *Hpa*I and *Eco*RI generates two α -specific fragments, as these enzymes cut outside and between the two α genes (Fig. 1A, B) (refs 2, 6 and E. Whitelaw, personal communication).

When E.J. DNA was digested in this way the two normal bands were present and of equal intensity, but in addition, in each case a third less intense band, 3.7 kilobases long, was produced. This fragment must have been derived from a third α

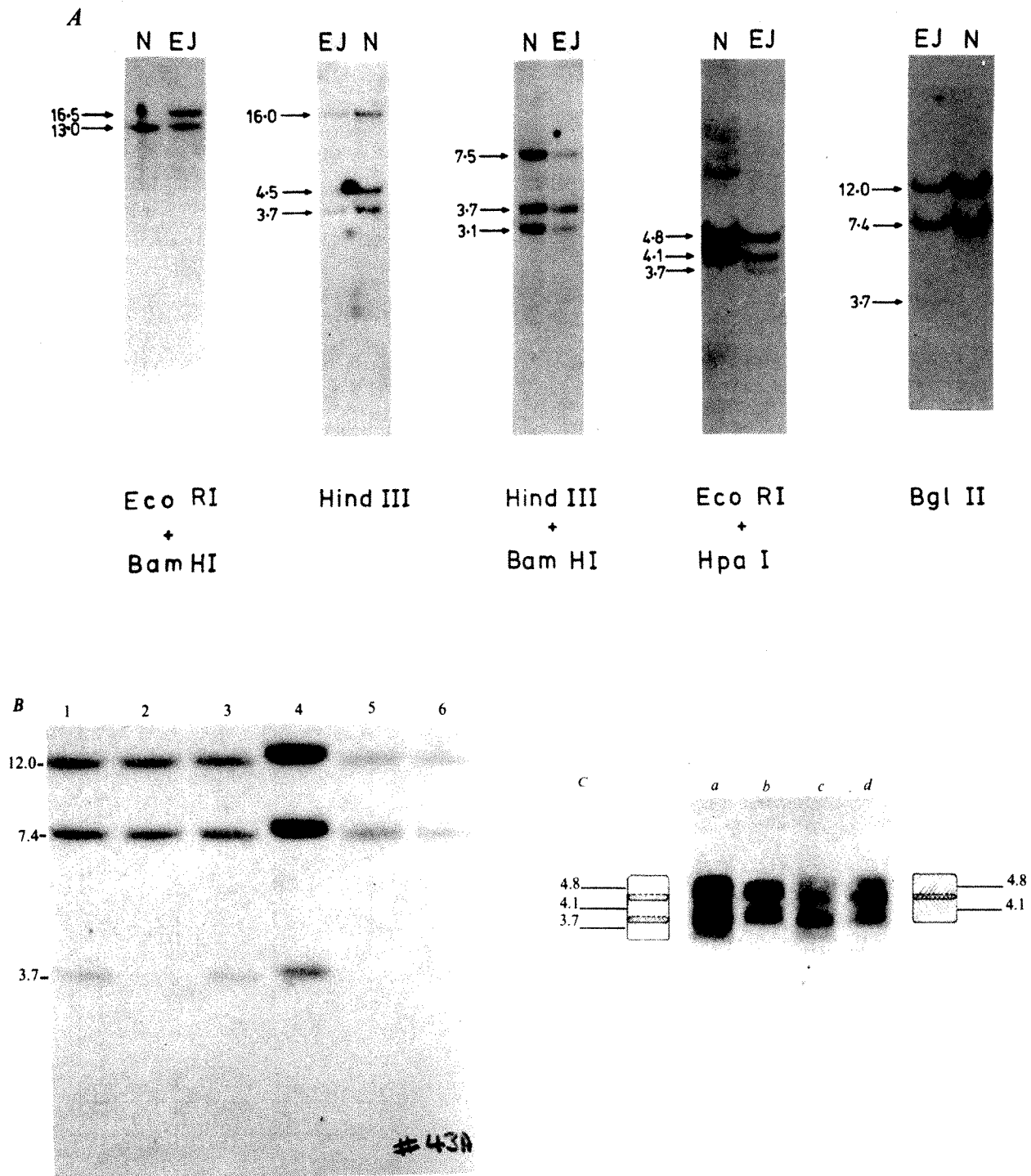


Fig. 1 A, Autoradiographs of DNA obtained from E.J. digested with various restriction enzymes. The sizes of DNA fragments are represented in kilobases. DNA was obtained by phenol chloroform extraction of peripheral blood or bone marrow⁹. Following restriction of 20 µg of DNA with 0.5–2.0 units of enzyme per µg DNA the samples were electrophoresed in 0.8% agarose and then transferred to nitrocellulose filters¹⁰. The DNA on the nitrocellulose filters was then hybridised to an α plasmid JW101 (ref. 11) which had been nick translated by the method of Maniatis *et al.* using ³²P-dCTP and ³²P-dATP (Radiochemical Centre) as radioactive labels¹². The filters were then washed in stringent conditions² and autoradiographed using Fuji-RX-X-ray film according to the method of Lasky and Mills¹³. An additional band (~10 kilobases) is present in the autoradiograph of the *Eco*RI/*Hpa*I double digest of normal DNA, representing an incomplete digest of the DNA which, in this case, was present in a greater amount than usual for such a digest. A faint (3.7 kilobase) band is present in the autoradiograph of the *Bgl*II digest of E.J. DNA in A. However, this band is clearly shown in B, lane 4. B, Autoradiographs of DNA obtained from D.J. (1), R.J. (2), C.J. (3), E.J. (4), normal (5), normal (6), which had been digested with *Bgl*II. The same pattern, consistent with the genotype $\alpha\alpha\alpha/\alpha\alpha$, was present in DNA from E.J., D.J. and C.J. The intensity of the normal fragments compared with the new 3.7-kilobase fragment is approximately 2:1, which is the ratio predicted for this gene arrangement. C, Autoradiograph of fragments produced by *Hpa*I digestion of DNA isolated from the abnormal 16.5-kilobase fragment generated by cleavage of E.J. DNA with a combination of *Eco*RI and *Bam*HI as described in the text. The 13-kilobase fragment (channels b, d) produced two α -gene fragments 4.8 and 4.1 kilobases long, whereas the 16.5-kilobase fragment produced three α -specific fragments of 4.8, 4.1 and 3.7 kilobases (channels a, c). The bands produced in these autoradiographs are broader than normal because when the samples, which were cut out from the low-melting temperature agarose, had been restricted and then re-applied to agarose gel, the DNA was in a larger volume than is conventionally applied to agarose gel for electrophoresis.

Table 1 Phenotype and genotype of R.J., D.J. and C.J.

	RBC ($\times 10^{12} \text{ L}^{-1}$)	Hb (g dl $^{-1}$)	Hct	MCV (fl)	MCH (pg)	Fe (mmol L $^{-1}$)	TIBC (mmol L $^{-1}$)	%HbA ₂	%HbF	α/β (counts)	α/β (S.A.)	α/β mRNA	Genotype
R.J.	4.8	14.2	0.399	82	29.5	21.5	88.5	2.50	0.67	1.01 1.18 1.06	1.03 1.06 1.24	2.5	$\alpha\alpha/\alpha\alpha$
D.J.	4.45	14.0	0.399	89	31.2	31.0	114.0	2.48	0.20	1.11 1.06 1.17	1.15 1.24 1.00	—	$\alpha\alpha\alpha/\alpha\alpha$
C.J.	4.70	14.3	0.409	86	30.4	19.0	79.5	2.47	0.29	1.17 1.16	1.00 0.99	2.9	$\alpha\alpha\alpha/\alpha\alpha$

Haematological and haemoglobin analysis was carried out using standard techniques⁷. All haematological studies were carried out on two separate occasions, with good agreement between duplicate results. E.J. had an unrelated haematological malignancy and therefore all studies were carried out on his haematologically normal sons R.J., D.J. and C.J. RBC, Red blood cell count; Hb, haemoglobin; Hct, haematocrit; MCV, mean corpuscular volume; TIBC, total iron-binding capacity; SA, specific activity.

gene present in the inserted DNA of the abnormal chromosome, with *Hpa*I and *Bgl*II sites 3.7 kilobases from their respective sites in the normal sequence between the two genes (Fig. 2). *Hind*III cuts normal DNA to the right and left within the α -globin genes^{2,5,6} to produce three fragments of 16, 4.5 and 3.7 kilobases. The 16- and 4.5-kilobase fragments are cleaved by *Bam*HI to yield 7.5- and 3.1-kilobase fragments, respectively. Digestion of E.J. DNA with *Hind*III generated only the three normal fragments (Figs 1A, 2). The 16- and 4.5-kilobase *Hind*III fragments were both cleaved by *Bam*HI to yield normal-sized fragments. The *Bam*HI sites on either side of the two α -globin genes are thus positioned normally, and a new *Hind*III site exists inside the inserted DNA sequence, giving rise to a new fragment 3.7 or 4.5 kilobases long and therefore indistinguishable from the normal *Hind*III fragments. The positions of the *Hpa*I, *Bgl*II and *Hind*III sites indicate that the inserted DNA is a repeat of intergenic DNA and an α -globin gene. A similar conclusion was drawn from a double digest of E.J. DNA with *Hpa*I and *Hind*III; four fragments were observed, all corresponding to those found in normal DNA⁶.

In Fig. 2, the α -gene fragments generated by various restriction enzymes are compared with those from a normal chromosome. Although all the abnormal fragments can be generated from an abnormal chromosome without a third α -globin gene locus in the inserted

DNA, the intensity of the autoradiograph bands in the *Bgl*II digest and the *Eco*RI/*Hpa*I digest suggested that the abnormal chromosome does contain a third α -globin gene. In both digests, band intensities agree with the normal to abnormal band ratio of 2:1 predicted in Fig. 2.

Finally, E.J. DNA was digested with *Eco*RI plus *Bam*HI and the products fractionated in a low-melting temperature agarose gel. The large 16.5-kilobase fragment was separated from the normal 13.0-kilobase fragment by slicing the gel between and outside the calculated band positions. Both DNA fragments were redigested with *Hpa*I and fractionated in a normal agarose gel. The 13.0-kilobase fragment produced two α -gene fragments of 4.8 and 4.1 kilobases as expected. However, the

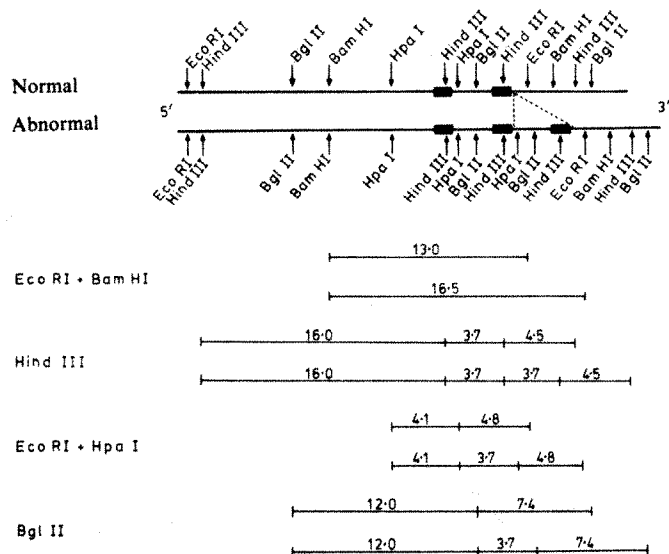


Fig. 2 The gene arrangement in E.J., C.J. and D.J. with two α genes on one chromosome and three on the other. The fragment sizes obtained from each chromosome following digestion with various restriction enzymes are shown below. The dotted lines indicate that the new arrangement contains an exact repeat of intergenic DNA and an α -globin gene. An identical map would be obtained if the inserted gene were placed to the right or left or between the normal duplicated α genes. The critical point is not the exact location of the inserted DNA but that it repeats precisely a part of the normal duplicated α -gene arrangement. As two fragments of equal size and one new fragment 3.7 kilobases long are produced by the enzyme *Bgl*II or a combination of *Hpa*I and *Eco*RI, the relative band intensity of 2:1 would be predicted from this model.

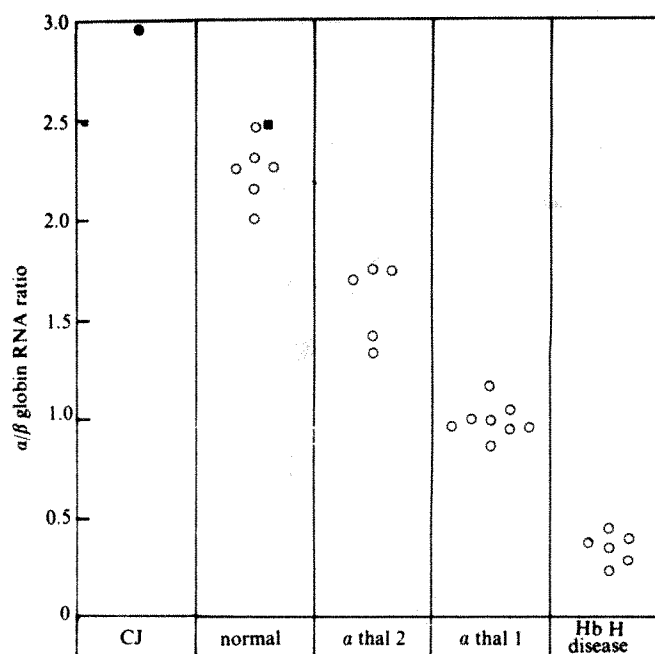


Fig. 3 α/β mRNA ratios in R.J. (■) and C.J. (●) compared with those of individuals with 4 (normal), 3 (α -thalassaemia 2; α thal 2), 2 (α -thalassaemia 1; α thal 1) and 1 (haemoglobin H disease; Hb H disease) functional α -globin genes. α/β mRNA ratios were determined as previously described¹. The range of α/β mRNA ratios in normal individuals was 2.0–2.45. This range of α/β -specific RNA sequences in the cells of normal individuals is higher than those previously reported and the significance of this is discussed elsewhere¹. However, the importance of these results does not lie in the absolute values obtained for α/β -specific sequences but rather the relative values in the various α -thalassaemia syndromes. The α/β mRNA ratio in C.J. with the arrangement $\alpha\alpha\alpha/\alpha\alpha$ was about 20% higher than normal and falls within the range that would be predicted by extrapolation from the other groups if there was no compensation in the transcription of mRNA in individuals with five α -globin genes.

16.5-kilobase fragment produced three α -gene fragments of 4.8, 4.1 and 3.7 kilobases (Fig. 1C), proving conclusively that the abnormal chromosome contains three α -globin gene sequences instead of two. Such a situation would arise if the abnormal chromosome had arisen by an unequal crossing over

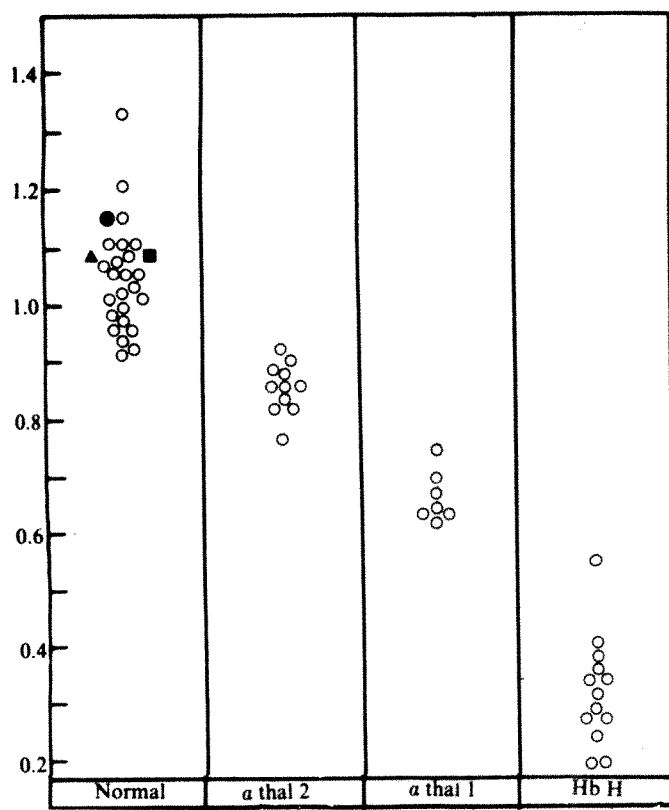


Fig. 4 α/β globin synthesis ratios in C.J. (●), D.J. (▲) and R.J. (■) compared with those individuals with varying numbers of functional α genes; α/β globin chain synthesis ratios were obtained as previously described¹⁴. The ratios obtained in 24 haematologically normal individuals, 11 subjects with α -thalassaemia 2, 7 subjects with α -thalassaemia 1, and 13 subjects with Hb H disease are shown. The ratios obtained in C.J., D.J. and R.J. are the average of two ratios obtained on separate occasions (Table 1) and fall within the range found in normal individuals.

between the α -globin genes, as proposed for the origin of the α -thalassaemia 2 genotype in several racial groups^{2,3}.

Haematological studies on D.J., C.J. and R.J. showed them to be normal (Table 1). Recently, α/β mRNA ratios in individuals with 4, 3, 2 and 1 functional α genes have been shown to fall into four non-overlapping groups¹. In R.J., who had the globin gene arrangement, $\alpha\alpha/\alpha\alpha$, the α/β mRNA ratio fell within the normal range (Fig. 3). However, in C.J., who had the $\alpha\alpha\alpha/\alpha\alpha$ globin gene arrangement, the α/β mRNA ratio was 20% higher than normal. Thus, it seems that when five α -globin genes are present, each is fully expressed at the mRNA level and there is no compensatory reduction in α mRNA output. However, the average α/β globin chain synthesis ratios in C.J. fell within the normal range (Table 1, Fig. 4).

Normal red cell precursors produce a slight excess of α chains, which are removed by proteolysis to achieve balanced α/β globin synthesis⁷. The fact that individuals with five α genes are haematologically normal and have no overall globin chain imbalance indicates that their red cell precursors can effectively compensate for an even greater excess of α -globin chain synthesis. However, the additional α gene might not always be functionally unimportant. Some cases of β -thalassaemia are unusually mild and there is genetic evidence that they are, in fact, compound heterozygotes for β -thalassaemia and 'silent β -thalassaemia', so called because it is not detectable in simple heterozygotes⁸. It is possible that some of these silent β -thalassaemias are, in fact, examples of the $\alpha\alpha\alpha/\alpha\alpha$ or $\alpha\alpha\alpha/\alpha\alpha\alpha$ genotype. Furthermore, one would predict that the basic thalassaemic nature of this genotype will only be revealed through its interactions with β -thalassaemia or β -chain haemoglobinopathies, such as Hb S.

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Aldridge for technical assistance, Dr B. Forget for plasmid JW101, and Dr C. Barton for bringing this family to our attention.

Note added in proof: Since this paper was submitted similar findings have been reported in individuals of Mediterranean background¹⁵.

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Coordinately expressed members of two chorion multi-gene families are clustered, alternating and divergently orientated

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The chorion (eggshell) of silkmoths consists of more than 100 distinct proteins. Individual components are synthesised in characteristic and overlapping sequence, at early, middle, late or very late developmental periods of choriogenesis (which lasts 2 days in total)^{1,2}. Protein sequencing^{3,4} indicates that components which belong to two size classes, A and B, are encoded by two respective families of evolutionarily homologous genes (multi-gene families). Genetic analysis in *Bombyx mori*^{5,6} has shown that chorion genes are tightly linked in three clusters on a single chromosome ($n = 28$). Total chorion mRNA of the wild silkworm, *Antheraea polyphemus*, has been copied into double-stranded cDNA and cloned by recombinant DNA procedures⁷. Many distinct cDNA clones have been selected and identified as members of the A and B multi-gene families by detailed cross-hybridisation⁷ and sequencing⁸ analysis. Stringent conditions which minimise cross-hybridisation permit use of these clones as specific probes. In many cases, we know the protein encoded and the developmental period when the clone sequence can be found in cytoplasmic RNA^{7,8}. Thus, we may now use these cDNA clones as probes to determine how genes of known developmental properties and evolutionary history are arranged within the chorion chromosomal region. We report here that two cloned, 14-kilobase chromosomal DNA segments contain multiple chorion genes. In each segment, the genes represent both A and B families, but only one developmental class: one segment contains only middle-period A and B genes and the other only late-period A and B genes. In both segments, members of the two families are arranged in alternating sequence and divergent orientation.

A library of *A. polyphemus* chromosomal DNA clones was established⁹, using the charon 4 derivative of phage λ as vector¹⁰. Screening of approximately 180,000 phage plaques with total chorion cDNA permitted selection of a 'bookshelf' of 175 independent chromosomal clones containing chorion genes. After plaque purification, these clones were spotted on lawns of

Escherichia coli strain CSH18 and re-screened¹¹, using as probes nick-translated, characterised cDNA clones. Chromosomal clones were selected which hybridised strongly with two non-cross-reacting cDNA clones, even in stringent conditions (80 °C and 0.45 M NaCl, or approximately 10 °C below T_m for that probe length). Clone APc110 hybridised with two late sequences: the A-family sequence, pc18, and the B-family sequence, pc401. By contrast, clone APc173 hybridised with two middle-period sequences: the A-family sequence, pc292, and the B-family sequence, pc10. This suggested that chorion genes are clustered according to the developmental period when they are expressed, rather than according to the multi-gene family to which they belong. Preliminary screening of the library with additional probes supported that suggestion (data not shown).

For detailed analysis of gene arrangements, restriction maps of APc110 and APc173 were constructed by standard procedures (Fig. 1). Blot-hybridisation¹² experiments revealed in

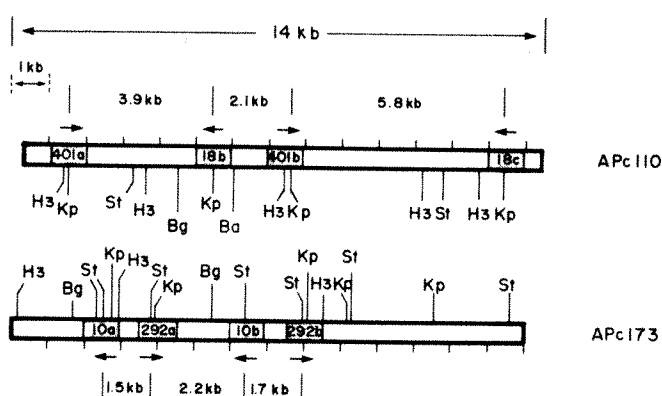


Fig. 1 Restriction maps of two chromosomal DNA clones from *A. polyphemus* carrying multiple chorion genes. Shading indicates the approximate location of genes numbered with reference to corresponding cDNA clones^{7,8}. Arrows show the direction of transcription. Clone APc110 contains only late-period genes, from two multigene families (pc18 = A; pc401 = B) and in opposite orientation. Clone APc173 contains only middle-period genes, again from two multi-gene families (pc292 = A; pc10 = B) and in opposite orientation. Locations of the genes were suggested by characteristic restriction sites known from the cDNA clones: *KpnI* for pc18, *SstI* for pc10, *KpnI* and *HindIII* for pc401, and *KpnI* and *SstI* for pc292. Confirmation came from detailed blot-hybridisation analysis using double enzyme digests. For example, the largest *KpnI* fragment of APc110 (~5.8 kilobases (kb)) contains both pc401 and pc18 sequences; after secondary digestion with *HindIII*, the left-most derivative (~3.6 kilobases) contains only pc401 sequences, the right-most derivative (~0.7 kilobases) contains only pc18 sequences, and the middle derivative (~1.5 kilobases) contains neither. Further confirmation came from blot-hybridisation experiments with 5'-specific or 3'-specific probes, which also defined the directions of transcription (see Fig. 2). One or more additional unmapped *SstI* sites exist between the two right-most *SstI* sites shown for APc173. Ba, *BamHI*; Bg, *BglII*; H3, *HindIII*; Kp, *KpnI*; St, *SstI*.

each case two non-contiguous copies of each of the represented cDNA sequences, in an alternating arrangement (BABA). Locations of the genes on the map were initially suggested by characteristic restriction sites known to be present in the clone cDNA sequences. These locations were verified by blot-hybridisation analysis of double enzyme digests (see Fig. 1 legend).

All four cDNA clones represented in these chromosomal segments have been sequenced. Thus, we could take advantage of centrally located restriction sites (*KpnI* for pc401 and pc18; *SstI* for pc10 and pc292) to generate probes representing either the 5' half or the 3' half of the corresponding mRNA. Blot-hybridisation of the chromosomal clones with these half-probes

defined the direction of transcription for each chromosomal gene (see, for example, Fig. 2). All experiments with half-probes gave a consistent directional map: A and B genes are transcribed in opposite orientations (arrows in Fig. 1), that is, A genes are transcribed from one DNA strand and B genes from the other.

The analysis with half-probes also established firmly that the blocks of each cDNA sequence represent two different gene copies, rather than one copy split in half by a very long intervening sequence. For example, in APc110, the pc401 half-sequences from left to right showed a 5'-3'-5'-3' progression, which is inconsistent with a single gene copy.

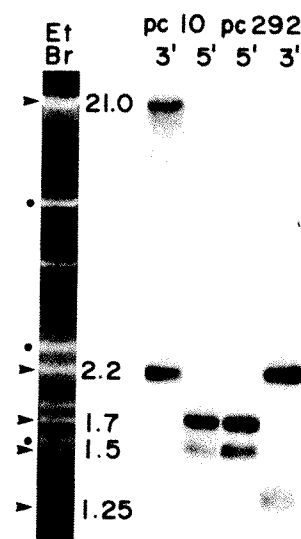


Fig. 2 Determination of transcriptional direction. The sequenced cDNA clones (ref. 8, unpublished observations, and S. G. Tsitilou, personal communication) were nick-translated and cleaved with *SstI* to generate separate probes specific for the 5' region or 3' region of each mRNA. Here blot-hybridisations of an *SstI* digest of APc173 are shown using such half-probes. The first track displays the ethidium bromide-stained total DNA pattern; arrowheads indicate DNA fragments which include chorion gene sequences (see the next four tracks), small dots indicate fragments which include moth DNA but no chorion sequences, and the unmarked fragments are derived solely from the chorion 4 vector. The blot-hybridisation patterns show that the 5' regions of both pc10 and pc292 are found in the same two fragments (1.5 and 1.7 kilobases), whereas both 3' regions are only found together in a third fragment (2.2 kilobases); the 3' regions of pc10 and pc292 are also found individually, in two fragments of approximately 21.0 and 1.25 kilobases, respectively. These results are only consistent with divergent orientation of the genes (arrows in Fig. 1).

Blot-hybridisations of the chromosomal clones with total chorion cDNA revealed no additional chorion genes other than those shown in Fig. 1. However, sequencing of the left-hand end of APc110 revealed the presence of a very short sequence corresponding to the 5'-untranslated region of another pc18 gene (data not shown). Thus, two divergent AB pairs are found in each chromosomal clone (18a, 401a; 18b, 401b; 10a, 292a; 10b, 292b). We suspect that the right-hand copy of pc18 (18c in Fig. 1) is indicative of a third pair in APc110. The pairs are not part of invariant repeats; for example, the two *KpnI* fragments which contain the 3' ends of genes are unequal in length (3.9 and 5.8 kilobases), and also differ in several restriction sites.

The genes represented in APc110 correspond to two of the most abundant chorion mRNA sequences¹³. Do additional copies of these genes exist in the genome, and if so, are all copies found as divergent AB pairs? Figure 3 provides affirmative answers to both questions. Total moth DNA was restricted with *KpnI* and blot-hybridised with 5'-specific or 3'-specific probes

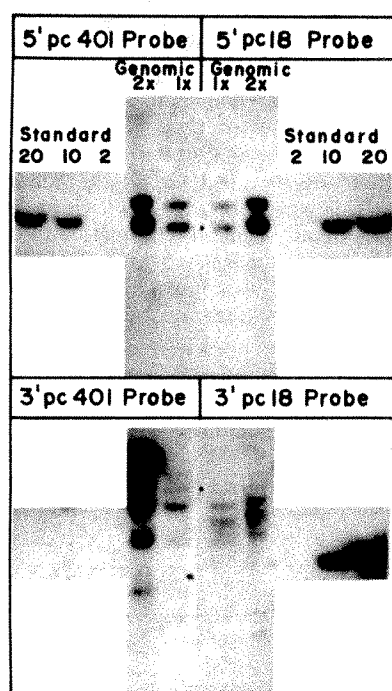


Fig. 3 Detection and organisation of repeated chorion genes in the moth genome. Total DNA from pooled developing pupae was digested with *KpnI*, electrophoresed, transferred to nitrocellulose¹² and hybridised with 5'- or 3'-specific ³²P-probes consisting of *KpnI* fragments from nick-translated cDNA clones pc401 and pc18; each probe contained the appropriate half of the chorion cDNA insert plus contiguous plasmid sequence. Hybridisation was at 75 °C in 0.45 M NaCl. Two aliquots of moth DNA were used in each experiment (1×, 10 µg; 2×, 20 µg). Reconstruction standards were included to estimate the gene multiplicity. They consisted of increasing amounts of a *HindIII/KpnI* digest of a subcloned *HindIII* fragment from APc110 (containing the entire *18b* gene and the 5' half of the *401b* gene; see Fig. 1), plus 10 µg sheared calf thymus DNA; only the hybridising *HindIII* to *KpnI* moth DNA fragments are shown. Each standard corresponds to the indicated number of gene copies per haploid genome ($C = 1$ pg), relative to the 1× moth DNA sample. Two major genomic bands hybridise with both 5' probes (top). When a mixture of the two probes is used, again only two bands are seen, confirming that the 5' ends of both types of genes hybridise to the same fragments (data not shown). The lower band (2.1 kilobases; dot) is expected from the map of APc110 (*KpnI* fragment containing the 5' ends of *18b* and *401b*; Fig. 1). The upper band (2.5 kilobases) indicates the existence of a second type of linked and divergent *401/18* pair in *A. polyphemus*. The combined intensities of the two bands, in comparison with the standards, indicate the existence of approximately 15 ± 5 *401/18* gene pairs per haploid genome (the gene *18* estimate is more dependable in this respect, as probe, standard and genomic fragment are co-terminous, whereas the standard lacks 130 of the 300 base pairs of *401* sequence represented in both probe and genomic fragment). At very long exposures, faint hybridisation to additional high molecular weight bands can be seen with both probes, possibly indicating limited polymorphism, single copies of additional *401/18* pairs, or cross-hybridisation with different but similarly organised genes of the same multi-gene families^{7,8}. Hybridisations with 3'-specific probes (bottom) reveal multiple bands, only some of which are identical for the two genes. This indicates that heterogeneity beyond the 3' ends of the genes is much greater than between the 5' ends. The expected 3.9-kilobase fragment (between *401a* and *18b*; Fig. 1) is indicated by a dot; the expected 5.8-kilobase fragment (between *401b* and *18c*) is seen in long exposures, but is smeared because of localised overloading of the gel (it co-migrates with a large number of unrelated *KpnI* genomic DNA fragments).

from cDNA clones pc401 or pc18, together with reconstruction standards. From the intensities of the blots, we estimate that approximately 15 ± 5 copies each of the *18* and *401* genes exist per haploid genome. With the 5' probes, only two major fragments hybridise, and they are the same for both *401* and *18*. The more intense fragment (which is found in approximately 10 copies per genome) corresponds to what is expected from clone APc110, whereas the other is longer by 0.4 kilobases. As these fragments were generated with *KpnI*, which cleaves within both *401* and *18* genes, and as each fragment contains the 5' ends of both *401* and *18*, we conclude that the multiple copies of these two genes are present in the genome as divergent AB pairs.

The map of APc110 shows length and restriction site variability 3' to the genes. As might be predicted, multiple bands of genomic DNA hybridise with each 3' probe, and some of these are different for the two probes. This confirms that greater variability is found in the long DNA segments containing the 3' ends of the genes than in the short DNA segments containing the divergent 5' ends. We conclude that APc110, and presumably also APc173, are representative: the multiple copies of four major chorion genes are arranged in pairs, each pair containing, in divergent orientation, coordinately expressed members of two different evolutionary families.

The co-expressed H3-H4 and H2A-H2B histone genes in *Drosophila* are arranged as divergent pairs within a repeat unit¹⁴, whereas in sea urchins the repeat includes only tandemly orientated genes¹⁵. Genes for the 70,000 molecular weight heat-shock protein are clustered in two loci, *87A* and *87C*, and include both tandemly orientated and divergent copies^{16,17}. Three apparently co-expressed ovalbumin-like genes are clustered in a tandem orientation¹⁸. Mammalian β -like globin genes are found in tandemly orientated pairs, arranged in progression according to their temporal expression¹⁹⁻²¹. Analysis of human deletions has shown that the developmental switching-off of one globin gene pair is controlled by a sequence element many kilobases away in the 3' direction (but located 5' to the next gene pair)¹⁹. Thus, coordinately expressed gene clusters may not be uncommon for eukaryotes. The chorion clusters are reminiscent of the histone genes in that they involve more than one sequence family. Clustering of co-expressed genes apparently does not reflect polycistronic transcription, because in some cases the genes are divergent, and because even the tandemly orientated globin genes seem to be transcribed individually^{22,23}. Although alternative interpretations are possible, clustering may be related to a gross level of regulation, such as availability for transcription in a particular type of differentiated cell, or at a certain developmental stage or physiological state, as a result of localised unravelling of chromatin²⁴; a visible manifestation of such a process might be the heat-shock-induced puffing of the *87A* and *87C* loci²⁵, each of which contains more than one gene copy. By contrast, divergent orientation might be related to finer-level regulation, such as precise temporal or quantitative coordination. In prokaryotes, divergent gene clusters are controlled by centrally located *cis*-acting regulatory elements²⁶; in addition, bi-directionally acting regulatory genes have been demonstrated in both prokaryotes and eukaryotes, although the transcriptional orientation of the flanking genes subject to regulation is not always known (reviewed in refs 26-28). Divergent organisation of repeated genes may also have interesting evolutionary implications: it may be that the unit of expansion or contraction in these multi-gene families is the gene pair, rather than individual genes. Functional interpretation of the intriguing gene arrangement reported here must await investigation of the generality of its occurrence among other chorion genes. It would be particularly interesting to know how extensive a chromosomal region limited to a single developmental period is, and whether it contains copies of more than two different genes.

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Recombination of human influenza A viruses in nature

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In 1977, a unique event occurred in the epidemiology of influenza when a virus of the influenza A (H1N1) subtype, similar to a virus that had occurred in 1950, reappeared and caused worldwide epidemics but did not replace the prevailing influenza A (H3N2) subtype¹⁻⁶. Consequently, the two viruses co-circulated throughout the world^{1,7} and mixed infection of some individuals with both virus strains was detected^{8,9}, raising the possibility that recombination between the two strains might affect the future epidemiological behaviour of influenza. Serological analysis of virus isolates from influenza outbreaks during the winter of 1978-79, however, failed to detect any antigenic hybrids (H3N1 or H1N2). The investigation described here, was therefore, undertaken to detect recombinants among recent isolates of the H1N1 and H3N2 serotypes, involving genes coding for other than the surface proteins by RNA-RNA hybridisation. We report here the genetic characterisation of recombinants of both antigenic types.

Sequence homologies of the genome RNA segments of influenza virus isolates were compared using a competitive RNA-RNA reassociation assay and by direct RNA-RNA hybridisation¹⁰. For the competitive reassociation assay double-stranded RNA probes were prepared by annealing ¹²⁵I-labelled individual RNA segments to homologous unlabelled cRNA. To

determine if a particular virus isolate contains an RNA segment related to that of a labelled reference RNA segment, the double-stranded RNA was melted and allowed to reanneal in the presence of increasing amounts of RNA from the virus isolate being tested. If the unlabelled viral RNA contains an RNA sequence identical to that of the ¹²⁵I-labelled segment, the unlabelled RNA will then compete for annealing sites on the complementary RNA and prevent the reannealing of the labelled RNA. If, however, the unknown virus RNA does not contain an homologous segment or contains one that is only partly homologous, then the RNA either would not compete for annealing sites on the cRNA or it would compete less efficiently and the labelled RNA would reanneal completely or partly with the cRNA.

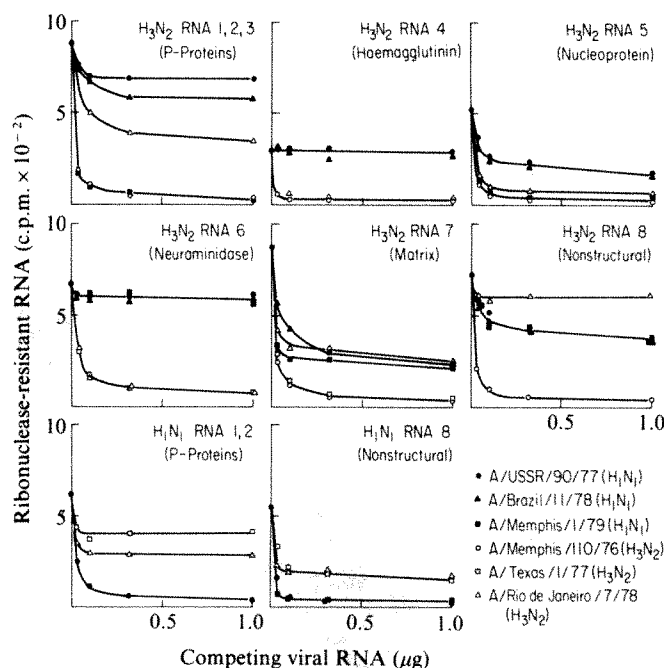


Fig. 1 Comparison of the RNA segments of recent influenza isolates with those of previous H3N2 and H1N1 strains by competitive hybridisation. The growth and purification of virus and the extraction of viral RNA has been described^{16,17}. Virion RNA (10 μg) from an H3N2 strain (A/Memphis/110/76) and an H1N1 strain (A/FW/1/50) was resolved into constituent genome segments by electrophoresis on a polyacrylamide-urea slab gel (22 × 14 × 0.15 cm) in 0.1 × Loenings buffer containing 7 M urea¹⁸, and 3% acrylamide cross-linked with 0.37% bis-acrylalcystamine¹⁹. This cross-linking reagent allows the gel to be solubilised by reduction. Electrophoresis was for 16 h at 25 °C with a constant voltage of 200 V. Following electrophoresis the gel was stained with ethidium bromide (5 μg ml⁻¹) and the RNA bands were visualised with a UV lamp and cut from the gel. The three largest RNA segments were not well resolved and were recovered in either one or two fractions. The gel slices were dissolved in 2 ml 0.05 M EDTA containing 10% 2-mercaptoethanol and 20 μg polyuridylic acid. (The poly-U acts as a carrier RNA during subsequent steps, but does not interfere with iodination¹⁷.) The RNA and acrylamide were precipitated and washed with ethanol, resuspended in 2.5 ml sodium acetate buffer (pH 5.0) and the RNA was electrophoretically separated from the acrylamide in an Iso electrophoretic concentrator cell, operated at 50 V for 2 h. The RNA was recovered in 0.2 ml and ethanol precipitated. Each RNA segment was iodine labelled using 800 μCi ¹²⁵I as described by Commerford²⁰. The iodinated RNA was further purified by cellulose chromatography to remove contaminating ¹²⁵I-labelled material²¹. Complementary RNA was extracted from infected canine kidney cells as described by Etkind and Krug²². Double-stranded (ds) RNA probes were made by annealing each ¹²⁵I-labelled RNA segment to an excess of homologous complementary RNA and then removing remaining single-stranded RNA by digestion with nuclease S1 as described by Hay *et al.*²³. Each competitive reassociation assay used a constant amount of a ¹²⁵I-labelled ds probe and varying amounts of unlabelled RNA extracted from the strains being compared with the reference RNA segments. Reactions were carried out in 15 μl 2.3 × SSC, 50% formamide²⁴. The mixtures were heated at 90 °C for 90 s and then allowed to reanneal at 70 °C for 40 h. Acid precipitable radioactivity was determined by liquid scintillation counting following digestion with ribonuclease A and T₁ (ref. 24).

Table 1 Homology between RNAs of reference influenza virus and isolates, detected by quantitative RNA-RNA hybridisation

Complementary RNA from:	% Homology of cRNA with ³² P-labelled virion RNA segments					
	1	2	3	5	7	8
Reference virus		P		NP	M	NS
A/Texas/1/77(H3N2)	100	100	100	100	100	100
A/USSR/90/77(H1N1)	66	44	54	62	76	78
Recent isolates						
A/Shanghai/9/79(H3N2)	97	(105)		94	99	100
A/Singapore/333/79(H3N2)	110	(102)		98	101	97
A/Brazil/11/78(H1N1)	59	27	53	55	82	77
A/California/10/78(H1N1)	93	103	107	74*	84	73
A/Texas/8/78(H1N1)	92	97	98	79*	74	75
A/Texas/10/78(H1N1)	100	91	108	74*	78	80

Quantitative RNA-RNA hybridisations were carried out by annealing individual ³²P-labelled virion RNA segments from A/Texas/1/77 with homologous or heterologous complementary RNA as described by Scholtissek *et al.*¹⁰. ³²P-labelled virion RNA from the two reference influenza strains was extracted from virus grown in chick embryo kidney cells in the presence of ³²P and the individual genome RNA segments were isolated following polyacrylamide gel electrophoresis as described previously¹⁴. Complementary RNA was isolated from infected, cycloheximide-treated, chick embryo fibroblast cultures using the method of Stephenson *et al.*¹⁵. The labelled RNA segments were annealed to an excess of homologous or heterologous complementary RNA in 2×SSC and then heated at 75 °C in 1×SSC, 1% formaldehyde before digestion with ribonuclease¹⁰. Values are expressed as per cent of counts protected in the homologous reaction. Genes 4 and 6 were not analysed by hybridisation because they could be readily identified by antigenic analysis. Values greater than 100% reflect the error inherent in measurements of this type. The standard error of the procedure determined by repetitive tests of different RNA segments is ±5%.

* Value <100% probably due to cross-contamination of NP RNA (segment 5) with RNA of N2 neuraminidase (segment 6).

Initial RNA comparisons were made using probes prepared with RNA segments isolated from an H3N2 virus of the Victoria serotype (A/Memphis/110/76) and an H1N1 strain, A/FW/1/50, previously shown to be nearly identical to the 'USSR' virus³⁻⁵. Figure 1 shows results of competitive reassociation assays of various virus strains with the H3N2 probes and two representatives of the H1N1 probes. RNA extracted from the homologous strain or closely related strains gave essentially complete competition of the labelled probes. The heterologous RNAs competed to varying degrees with the different RNA segments. There was, for example, little cross-competition with the genes coding for the surface proteins of the prototype strains (genes 4 and 6), while genes 5 and 7 (nucleoprotein and matrix) showed much more cross-competition, indicating a greater conservation of the base sequence coding for these internal proteins, in agreement with the earlier findings of Scholtissek *et al.*⁴. In all cases, however, competition with RNA derived from the heterologous prototype strain was clearly distinguishable from the homologous competition.

One strain shown in Fig. 1, A/Memphis/1/79 (a recent H1N1 isolate), is clearly a recombinant, as four genes (1, 2, 3 and 5) coding for the three 90,000-molecular weight 'P' polypeptides and for the nucleoprotein are homologous with those of the H3N2 strain, while those coding for the surface proteins and genes 7 and 8, coding for the matrix and non-structural proteins, are homologous with those of the H1N1 strain. Comparable results (Table 1) were obtained by directly hybridising ³²P-labelled gene segments isolated from A/Texas/1/77 (H3N2) with complementary RNA isolated from cells infected with A/California/10/78 (H1N1) and other isolates from early outbreaks of influenza during the winter of 1978-79 in the US (A/Texas/8/78 and A/Texas/10/78). Three other H1N1 isolates obtained during the winter of 1978-79 (A/California/10/78; A/Berkeley/40/78; A/Georgia/64/78) were also tested by competitive hybridisation and shown to be identical recombinants (Table 2).

Many of the recent H1N1 strains mentioned above, which were shown to be recombinants between previous H1N1 and H3N2 viruses, are antigenically indistinguishable when tested with ferret sera or monoclonal antibodies (unpublished data) from A/Brazil/11/78, the prototype for a minor variant of the USSR strain predominant in South America during 1978 (ref.

11). Analysis of the RNAs of this prototype variant (Fig. 1, Table 1) indicated, however, that all of its gene segments were derived from an H1N1 strain.

To determine if the recent circulation of recombinant influenza viruses was limited to the above described H1N1 strains, the genetic composition of some recent H3N2 isolates¹² was also analysed. No evidence was found for the presence of genes of H1N1 origin in H3N2 strains isolated in south-east Asia in 1979 (Table 1). A South American H3N2 virus isolate (A/Rio de Janeiro/7/78), however, proved to have a different genetic composition. When RNA from this strain was tested in competitive reassociation assays (Fig. 1), only genes 4 and 6, coding for the surface proteins, of the H3N2 strain were shown to have homologous counterparts in this virus. The RNA did not contain sequences homologous to the other genes of either the H1N1 or H3N2 prototypes, although gene 5 (nucleoprotein) seemed to be more closely related to the corresponding H3N2 gene than to that of H1N1.

In an effort to determine the origin of the genes coding for the non-surface proteins of this virus, ¹²⁵I-labelled probes were prepared from its RNA segments and these were used in competitive reassociation assays with RNA from a series of 20 viruses. These included representatives of the major human influenza A viruses and selected strains from lower animals, as well as an influenza B virus. Mixtures of H3N2 and H1N1 RNA were also tested to rule out the possibility that this virus might actually be a mixture with two copies of each gene. With each segment only RNA from A/Rio de Janeiro/778 was effective in competition assays. None of the other strains tested were found to contain completely homologous RNA.

Our findings that the H1N1 virus prevalent in the US during the winter of 1978-79 contained four genes from an H3N2 virus, are similar to those of Young and Palese¹³, who studied these viruses by oligonucleotide and peptide mapping. Evidently, this recombinant had a distinct selective advantage over the parental strains. Possibly, the immune status of the population favoured the surface proteins of the H1N1 variant, while the replication complex of the H3N2 strain was better adapted for infection and transmission. The earliest examples of these recombinant viruses so far obtained were isolated in November and December of 1978 (Table 2). As these viruses

Table 2 Summary of gene derivation of recent H1N1 and H3N2 virus isolates analysed

	RNA segments							
	1	2	3	4	5	6	7	8
	P			HA	NP	NA	M	NS
H1N1 strains								
A/Brazil/11/78 8 May 78	H1	H1	H1	H1	H1	H1	H1	H1
A/Berkeley/40/78 29 Nov 78								
A/Calif/10/78 10 Dec 78								
A/Texas/8/78 28 Nov 78								
A/Texas/10/78 12 Dec 78	H3	H3	H3	H1	H3	H1	H1	H1
A/Memphis/1/79 2 Feb 79								
A/Ga/64/79 19 Feb 79								
H3N2 strains								
A/Rio de Janeiro/7/78 8 May 78	?	?	?	H3	?	H3	?	?
A/Singapore/333/79 7 April 79								
A/Shanghai/9/79 16 April 79	H3	H3	H3	H3	H3	H3	H3	H3

H1, RNA segment homologous with corresponding gene of A/USSR/90/77 (H1N1).

H3, RNA segment homologous with corresponding gene of recent H3N2 isolates of the Victoria or Texas serotypes.

?, RNA segment not closely related to H1N1 or H3N2 strains or any other influenza strain yet tested.

are antigenically identical to the H1N1 variant, which was isolated about 6 months earlier, our finding that A/Brazil/11/78 contained all the genes from the USSR strain indicates that the drift of the H1 haemagglutinin from A/USSR/80/77 to A/Brazil/11/78 has occurred independently of recombination.

The significance of the finding that the H3N2 virus A/Rio de Janeiro/7/78, which contains genes coding for surface proteins identical to those of other recent H3N2 isolates, but derives all other genes from an unknown source remains uncertain. This isolate was chosen for study primarily because it came from an outbreak in the same country and in the same week as A/Brazil/11/78, and therefore was of interest to us as a potential recombinant with H1N1 genes. This virus and others from the outbreak also have the unusual property of cross-reacting equally with both the Texas and Victoria serotypes, as recently found for a small number of other H3N2 isolates⁷. It was isolated in chicken embryos and egg passaged several times before use in this study. Neither the hybridisation results nor subsequent cloning have given any evidence that it is a mixture of viruses. The unknown genes of A/Rio de Janeiro/7/78 may have been derived from a previously unrecognised human influenza virus or from some animal influenza that was not tested

in the experiments described here. In either case, the identity of only the haemagglutinin and neuraminidase genes of A/Rio de Janeiro/7/78 with those of recent H3N2 strains suggests that this unusual virus may be a further example of a recombinant virus infecting man.

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Note added in proof: The unusual H3N2 virus strain described above (A/Rio de Janeiro/78) has recently been analysed by J. Young and P. Palese and shown to be genetically identical to a laboratory-derived recombinant of a Victoria strain in the A/PR/8/34 strain (personal communication). This suggests that A/Rio de Janeiro/78 may not be a natural "recombinant". We cannot be certain, however, whether the isolation of this virus and several other identical isolates was due to actual infection with a laboratory-derived strain or to laboratory contamination of clinical samples. Epidemiological studies to resolve this question are underway.

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***E. coli* RNA polymerase promoters on superhelical SV40 DNA are highly selective targets for chemical modification**

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Supercoiled DNAs are required or preferred in a number of biological processes such as repair and transcription. This requirement may be due to special properties which are present in supercoiled (FI) DNAs but not in the corresponding allomorphic forms. If sufficiently supercoiled, FI DNAs seem to contain single-stranded regions that are accessible to single-strand specific reagents and endonuclease¹⁻³. The free energy associated with superhelix formation may also be used to drive ligand-DNA or protein-DNA interactions³⁻⁷. *In vitro* studies have shown that superhelical DNA is a better template for transcription than its corresponding allomorphic forms⁸⁻¹⁵, and this is due probably to a change in the initial binding affinity of the RNA polymerase at promotor sites¹⁵⁻¹⁸. Previous work in our laboratory has suggested that the single-strand specific probe *N*-cyclohexyl-*N'*-β-(4-methylmorpholinium) ethyl carbodiimide (CMC), which almost totally inhibits transcription of SV40 and PM2 DNA^{14,18}, is acting preferentially at promotor sites¹⁸⁻²⁰. We report here experiments to determine the selectivity of CMC for promoters on superhelical SV40 DNA. After limited reaction with CMC there was substantially less *Escheri-*

chia coli RNA polymerase bound to reacted SV40 DNA than to the unmodified DNA. Marked inhibition of transcription from the modified DNA demonstrated that CMC is highly selective for *E. coli* RNA polymerase promoters in superhelical DNA, suggesting that the introduction of superhelical turns may alter the parameters by which the enzyme interacts with DNA through promotor recognition and opening of the DNA.

E. coli RNA polymerase was bound to supercoiled SV40 DNA at concentrations exceeding those for saturation as determined by previous experiments¹⁵. Complexes were cross-linked, purified and cleaved with *Hpa*II (Fig. 1). *Hpa*II endonuclease cleaves SV40 at 0.735 map units; this area does not contain any *E. coli* RNA polymerase binding sites¹⁵. Typical SV40 DNA complexes with *E. coli* RNA polymerase are shown in Fig. 1. This figure also demonstrates the efficient removal of excess polymerase and glutaraldehyde by Sepharose 6B column chromatography (full details will be published elsewhere). *E. coli* RNA polymerase binding sites were precisely mapped on over 400 DNA-RNA polymerase complexes in three separate experiments. Figure 2 gives map measurements of ~200 molecules in one experiment. *E. coli* RNA polymerase binding sites were consistently observed at positions 0.13, 0.18-0.21, 0.47, 0.52-0.53, 0.60-0.62, 0.65, 0.82, 0.925 and 0.975 map units on an SV40 map using the single *Eco*RI cleavage site as map position 0.0. As the number of bound polymerase molecules increased, it became more difficult to spread the DNA sufficiently for this type of map measurement. Most complexes containing nine molecules of RNA polymerase per DNA molecule were observed in a collapsed form due to polymerase-polymerase interactions. Therefore, most molecules used in map measurements contained five or six polymerase per DNA molecule. However, the localisation of binding sites on a subpopulation of molecules that contained nine molecules per

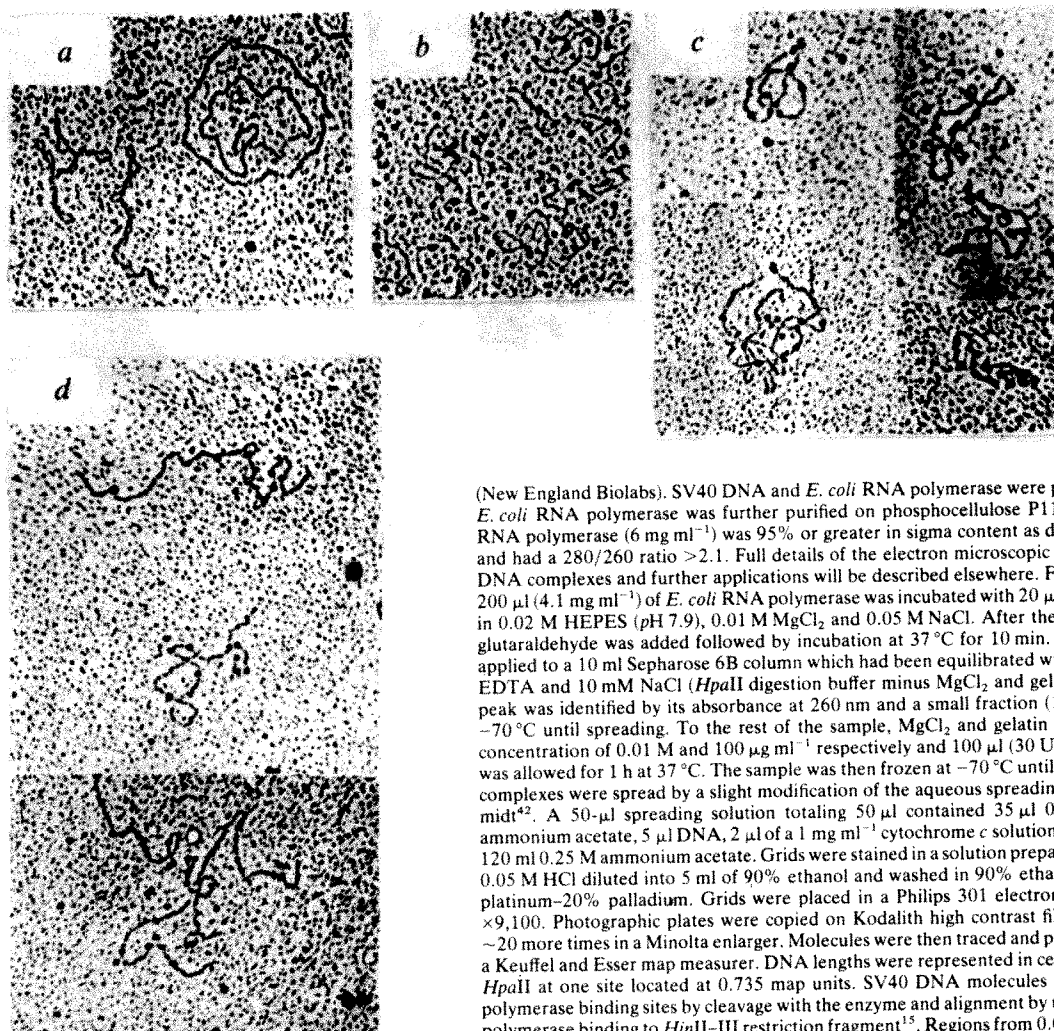


Fig. 1 Enhanced visualisation of glutaraldehyde-fixed RNA polymerase-SV40 DNA complexes by removal of excess RNA polymerase and fixative using Sepharose 6B column chromatography. Electron micrographs of: *a*, superhelical SV40 DNA FI; *b*, *E. coli* RNA polymerase-SV40 DNA complexes before Sepharose 6B chromatography; *c*, *E. coli* RNA polymerase-SV40 DNA complexes after Sepharose 6B chromatography; *d*, *E. coli* RNA Polymerase-SV40 DNA complexes cleaved with *HpaII* restriction endonuclease

(New England Biolabs). SV40 DNA and *E. coli* RNA polymerase were prepared as described previously¹⁵. *E. coli* RNA polymerase was further purified on phosphocellulose P11 as described previously⁴⁰. *E. coli* RNA polymerase (6 mg ml⁻¹) was 95% or greater in sigma content as determined by rifampin inhibition⁴¹ and had a 280/260 ratio >2.1. Full details of the electron microscopic visualisation of RNA polymerase-DNA complexes and further applications will be described elsewhere. For binding of polymerase to DNA, 200 μ l (4.1 mg ml⁻¹) of *E. coli* RNA polymerase was incubated with 20 μ g of SV40 DNA at 37 °C for 30 min in 0.02 M HEPES (pH 7.9), 0.01 M MgCl₂ and 0.05 M NaCl. After the 30 min incubation, 6.25 μ l of 8% glutaraldehyde was added followed by incubation at 37 °C for 10 min. The sample was then immediately applied to a 10 ml Sepharose 6B column which had been equilibrated with 10 mM HEPES (pH 7.4), 1 mM EDTA and 10 mM NaCl (*HpaII* digestion buffer minus MgCl₂ and gelatin). The DNA-RNA polymerase peak was identified by its absorbance at 260 nm and a small fraction (100 μ l) was removed and frozen at -70 °C until spreading. To the rest of the sample, MgCl₂ and gelatin (autoclaved) were added to a final concentration of 0.01 M and 100 μ g ml⁻¹ respectively and 100 μ l (30 U) of *HpaII* was added and digestion was allowed for 1 h at 37 °C. The sample was then frozen at -70 °C until DNA and DNA-RNA polymerase complexes were spread by a slight modification of the aqueous spreading technique described by Kleinschmidt⁴². A 50- μ l spreading solution totaling 50 μ l contained 35 μ l 0.001 M EDTA (pH 7.5), 5 μ l 5 M ammonium acetate, 5 μ l DNA, 2 μ l of a 1 mg ml⁻¹ cytochrome *c* solution. The hypophase solution contained 120 ml 0.25 M ammonium acetate. Grids were stained in a solution prepared with 5 μ l 0.05 M uranyl acetate, 0.05 M HCl diluted into 5 ml of 90% ethanol and washed in 90% ethanol, dried and shadowed with 80% platinum-20% palladium. Grids were placed in a Philips 301 electron microscope and photographed at $\times 9,100$. Photographic plates were copied on Kodalith high contrast film (Eastman-Kodak) and enlarged ~ 20 more times in a Minolta enlarger. Molecules were then traced and polymerase positions measured using a Keuffel and Esser map measurer. DNA lengths were represented in centimetres. SV40 DNA is cleaved by *HpaII* at one site located at 0.735 map units. SV40 DNA molecules could be orientated for location of polymerase binding sites by cleavage with the enzyme and alignment by using the information obtained from polymerase binding to *HinII*-III restriction fragment¹⁵. Regions from 0.05 to 0.01, 0.03 to 0.04, 0.65 to 0.75

and 0.93 to 0.965 map units did not contain polymerase binding sites¹⁵. This allowed only one possible orientation for essentially all of the molecules that were measured. The number of polymerase molecules bound to each DNA molecule was counted directly from the photographic plates. Before spreading, relaxed circular SV40 DNA was added to DNA-RNA polymerase complexes in a concentration of 0.5 μ g ml⁻¹. This DNA acted as a marker for determining full-length DNA-RNA polymerase complexes for mapping of polymerase binding sites. At this concentration, at least 4 or 5 measurable circular molecules of SV40 DNA were found on each photographic plate.

DNA molecule and were sufficiently spread for map measurement gave a similar result (data not shown).

The total number of *E. coli* RNA polymerase molecules bound to each SV40 FI DNA molecule was determined for over 400 complexes at saturating concentrations of *E. coli* RNA polymerase (see Fig. 3a for data on molecules used for map measurements in Fig. 2). A maximum was observed at nine polymerase molecules per DNA molecule. The results indicate that in the supercoiled form of SV40 DNA there are nine specific RNA polymerase binding sites which may be occupied at saturating concentrations of the enzyme. No preferential binding to any particular site was observed, although increased stability from dissociation has been observed for the binding sites at 0.13 and 0.18-0.21 map units (P. Hale, unpublished observation).

The apparent equilibrium reactivity of the single-strand regions in the superhelical form is between 108 and 121 CMC molecules in the conditions used^{19,21}. No detectable reaction of nicked SV40 DNA was observed and saturating ethidium bromide density measurements did not detect any significant decrease in superhelical density²¹.

We have studied polymerase binding to, and template activity of, SV40 FI DNA which has been modified by 7 and 15 min of CMC reaction. The mean extent of carbodiimide binding after 15 min of reaction is 10.7 molecules CMC per molecule SV40 as determined by buoyant density analysis²² (Fig. 4). The linear nature of the kinetics allows interpolation to 7 min of reaction;

after fitting the data to a straight line, the mean reactivity at 7 min was found to be 5.0 CMC molecules per SV40 DNA molecule.

Evaluation of the extent of RNA polymerase binding by electron microscopy as well as the inhibition of transcription in

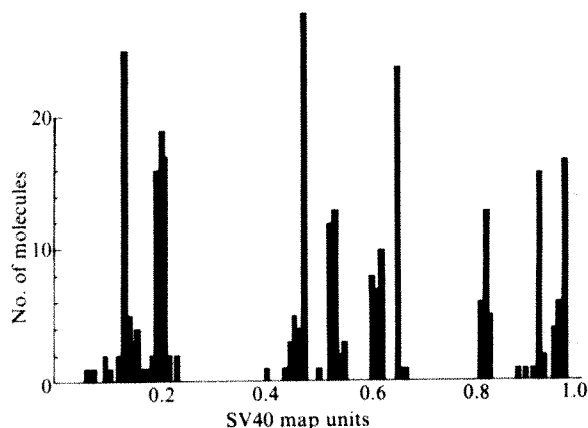


Fig. 2 Map location of *E. coli* RNA polymerase binding sites on native SV40 DNA FI as determined by electron microscopy. Samples were prepared as described in Fig. 1.

Table 1 Comparison of RNA polymerase binding and transcription between untreated SV40 FI DNA and 7 and 15 min CMC-reacted DNA

A	B	C	D	E	F	G
CMC reaction (min)	Moles CMC per mole DNA	Mean moles polymerase per mole DNA	Moles CMC per polymerase binding site	% Inhibition of polymerase binding	% Inhibition of transcription -Rif	% Inhibition of transcription +Rif
0	0	7.1	0	0	0	0
7	5.0	—	0.56	—	35%	54%
15	10.7	4.5	1.2	37%	61%	88%

A, SV40 DNA I (25 μ g) was reacted with a 500 molar excess of CMC and purified from excess reagent as previously described¹⁸. B, The molecules of CMC bound per molecule of SV40 DNA were determined by buoyant density analysis as shown in Fig. 4. The 7-min reaction was interpolated from the data. C, The mean number of RNA polymerase molecules per SV40 DNA was determined from the data in Fig. 3. D, The number of CMC molecules per RNA polymerase site was calculated by dividing column B by nine, the total number of RNA polymerase binding sites on untreated SV40 DNA. E, The per cent inhibition of binding was calculated from the data of column C. F and G represent transcription assays of RNA polymerase-SV40 DNA complexes before preparation for electron microscopy. Transcription assays were performed as previously described^{15,18}, with DNA and polymerase incubated together for 10 min before the addition of nucleotide triphosphates (NTP). In transcription assays containing CMC-reacted DNA, DNA was incubated in transcription buffer 10 min before polymerase addition as previously described¹⁸. CMC reactions were repeated 4 times with per cent inhibition of transcription, with or without rifampin, showing reproducibility of $\pm 2\%$. Rifampin, 4 μ g ml⁻¹, was added with NTP in column G.

the presence and absence of rifampin should reveal whether extremely limited CMC modification is specific for promoter sites. The electron microscopy technique described was used to study the effects of 15-min CMC modification of the DNA on RNA polymerase binding (Fig. 3b). Maximum binding of RNA polymerase was observed at five polymerase molecules per DNA molecule. This loss of RNA polymerase binding is compared with the inhibition of transcription in Table 1. It can be seen that an average of 1.2 CMC molecules per polymerase site is sufficient to produce a 37% loss of binding as judged by electron microscopy (Table 1, D, E). Transcription inhibition of 61 and 88% occurred in the absence and presence of rifampin respectively (Table 1, F, G). Although electron microscopic mapping was not carried out on the 7 min CMC-reacted DNA (5.0 CMC per SV40 DNA) the lower inhibition of transcription is consistent with the data obtained for the 15-min CMC-reacted DNA (10.7 CMC per SV40 DNA). These results show that the *E. coli* RNA polymerase promoters on SV40 DNA FI exist in a state, or states, readily accessible to extremely limited modification by a reagent highly selective for unpaired bases. Rifampin resistance measures the capacity to initiate rapidly a ternary complex for transcription. Previous work with native SV40 DNA has shown that there is essentially no difference in transcription with or without rifampin¹⁵. Here we observe that on very limited CMC modification rifampin causes a further decrease in transcription of about 20% (Table 1, F, G) compared with transcription without rifampin. Hence it seems that CMC modification enhances rifampin sensitivity by weakening promoters. This is supported by earlier data for 2-h (CMC-reacted) SV40 DNA tested for rifampin sensitivity. In these experiments, sensitivity to the drug was measured by differences in template saturation at different rifampin concentrations¹⁸. The saturation point was decreased by 26 and 47% for 2 and 4 μ g ml⁻¹ rifampin, respectively. Unmodified SV40 DNA FI is insensitive to increase in rifampin from 2 to 8 μ g ml⁻¹ (ref. 15). The capacity of CMC to weaken or block RNA polymerase binding could depend on the nature of individual promoters. This has been tested by mapping the binding sites for one of the modified SV40 DNA samples.

Figure 5 shows the results of mapping RNA polymerase molecules bound to 15 min CMC-reacted SV40 FI DNA. When these results are compared with those for the untreated DNA (Fig. 2), it can be seen that the location of the sites is very similar on both the CMC-reacted and unmodified DNA. Nine sites are observed at map positions 0.11, 0.18–0.19, 0.46, 0.54, 0.60, 0.63, 0.83, 0.925 and 0.98. The binding sites were observed to be more heterogeneous on the CMC-reacted DNA, this may be due to the decrease in rifampin-resistant tight binding sites on the CMC-modified DNA. This would be anticipated if CMC modification in or near a given promoter could occur at different locations leading to a variation in binding position around the promoter site. No specific population of sites was observed to be particularly sensitive to CMC reaction or preferentially lost by a 15-min reaction of the DNA with CMC.

Also note that CMC modification increases RNA polymerase binding around the 0.30 and 0.40 map positions to a small extent. Recent assays of unmodified SV40 DNA restriction fragments retained on nitrocellulose filters by RNA polymerase show that these regions do contain weak affinity for the enzyme (P.T. Chan, unpublished observation).

The use of a slow-reacting reagent^{19,21} (108–124 molecules CMC/SV40) and only short reaction times has allowed us to limit the reaction to approximately one CMC per promoter. This situation precludes propagation of denaturation into RNA polymerase binding regions by modification at neighbouring sites. No significant loss of superhelical turns can occur with only

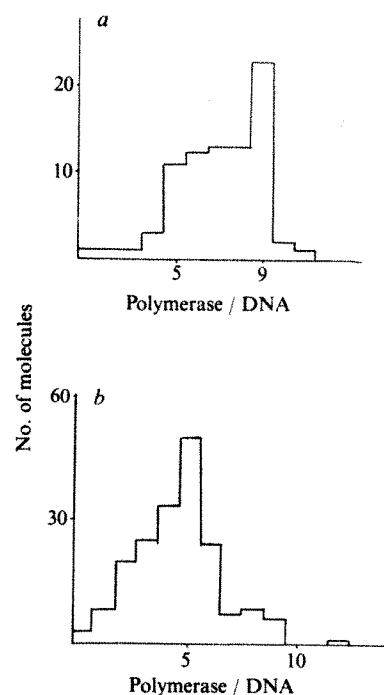


Fig. 3 The respective distributions of the number of *E. coli* RNA polymerase molecules per SV40 FI DNA for: a, unmodified; and b, 15 min. CMC-modified DNA. Supercoiled SV40 FI native or CMC-modified DNA was incubated with *E. coli* RNA polymerase, cross-linked with glutaraldehyde, chromatographed on Sepharose 6B and cleaved with *Hpa*II as described in Fig. 1. The resulting complexes were then spread for electron microscopy as described. The number of RNA polymerase molecules per DNA molecule were counted directly from the photographic plates. The reaction of SV40 FI DNA with CMC was performed at a 500-fold molar excess of CMC to nucleotides for 15 min at 37°C and excess reagent was removed by chromatography on BioGel A5m as described previously¹⁸. CMC-modified DNA was incubated with bovine serum albumin (0.5 mg ml⁻¹) for 10 min at 37°C before polymerase addition to absorb residual CMC¹⁸.

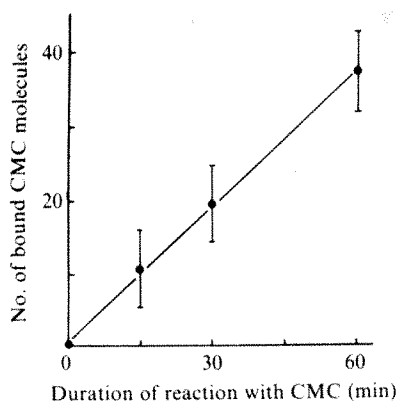


Fig. 4 Extent of CMC modification of SV40 DNA I as a function of time. The amount of CMC bound in the conditions described in Fig. 3 was evaluated using the equation of Bauer and Vinograd²²

$$\nu' = \frac{\theta(\bar{\nu}_3 + \Gamma') - (1 + \Gamma')}{(1 - \bar{\nu}_4\theta)}$$

where ν' is g of reagent bound per g of DNA; θ , the buoyant density of the modified DNA; $\bar{\nu}_3$, the partial specific volume of anhydrous DNA (0.479); Γ' , the net hydration of the modified DNA; and $\bar{\nu}_4$ the partial specific volume of CMC (0.814). Γ' was evaluated by the equations developed by Bauer and Vinograd²². The moles of CMC per mol of nucleotide was obtained by multiplying ν' by 430/423, the respective molecular weights of a Cs nucleotide and CMC. The buoyant density of SV40 DNA was determined from a G-C content of 0.4067 from the full sequence data²⁸ using the relationship $\theta = 0.0988 (G-C) + 1.6541$. This gives a θ value for SV40 DNA of 1.6944 g cm^{-3} . The above unpublished equation for θ against (G-C) is relative to a θ value for *E. coli* DNA of 1.7035 g cm^{-3} (ref. 43) and simply modifies the intercept of previous equations⁴⁴. Analysis of buoyant density was carried out by the method of Vinograd and Hearst⁴⁴ using *Micrococcus lysodeikticus* DNA as a marker. The θ value for the latter was found to be 1.7271 g cm^{-3} relative to SV40 DNA. Buoyant densities were determined in a Beckman model E analytical ultracentrifuge. All experiments were carried out at 40,000 r.p.m. and 20°C using a solvent consisting of 10 mM Tris, 1 mM Na_3EDTA (pH 8.0) and the appropriate amount of CsCl (Metallgesellschaft). Cells were scanned in conditions which yielded a magnification factor of ~ 19 . The chart recording was measured with a precision ruler (Keuffel and Esser). The total uncertainty in the measurement of the marker and sample peaks on the scanner trace was $\pm 0.04 \text{ cm}$, corresponding to an experimental precision of θ of $\pm 0.0003 \text{ g cm}^{-3}$, equivalent to ± 5.4 CMC molecules per molecule of DNA. The determination of θ is the major source of experimental error as transcription studies have shown that there is no greater than 2% difference between identical repeat reactions.

11 CMC molecules per SV40 DNA, thus excluding the possibility that CMC modification removes sufficient superhelical turns to reduce the torsional forces that may be responsible for structural alterations in promoters. Several models could explain why *E. coli* RNA polymerase binding sites are highly selective targets for CMC modification: (1) Supercoiling produces open promoters of single-strand character, allowing enhanced binding of RNA polymerase. This would explain the extreme sensitivity of promoters to CMC modification, as reactivity occurs at the imino sites of thymine and guanine and requires unpaired bases; (2) The promoter regions are made highly reactive due to structural transitions that are not presently understood; (3) CMC reactivity that occurs by either of the above models may be localised at sites adjacent to the promoter and perturb structural transitions that a promoter must undergo for appropriate recognition by RNA polymerase. This action at a distance or telestability²³ may be due to the CMC-induced disruption of "barriers, sinks or sources of sequence-specific conformational preference"²⁴ that may be needed to produce the recognition site for RNA polymerase.

Support for the localisation of altered secondary structure comes from a very recent study of Shishido²⁵ using S_1 endonuclease to map preferential unpaired or weak duplex regions in SV40. The cleavage sites were found at 0.15, 0.28, 0.38, 0.39, 0.44, 0.56, 0.89 and 0.98 SV40 map units. The average distance of an RNA polymerase molecule from a respective S_1 site is 0.032 map units (167 base pairs). Given an

uncertainty of ± 0.02 map units (103 base pairs) for the assignment of RNA polymerase binding sites by electron microscopy leads us to conclude that six of the S_1 sites are in close proximity to RNA polymerase binding regions.

If a promoter constitutes approximately 60 base pairs²⁶ one would anticipate that CMC modification would produce a differential effect on binding dependent on its location, that is directly in the promoter sequence or at adjacent sites upstream or downstream. This would eliminate binding in some cases but only weaken binding in others. However, if initiation is dependent on a precise RNA polymerase-DNA interaction one would anticipate a more drastic effect on transcription. This seems to be the case (Table 1) and is supported by our previous observation that rifampin sensitivity ($2-8 \mu\text{g ml}^{-1}$) is not seen until SV40 FI DNA is modified with CMC¹⁸. Hence one CMC per promoter produces a 37% loss of binding which represents an 88% decrease in transcription in the presence of $4 \mu\text{g ml}^{-1}$ rifampin.

All the RNA polymerase sites appear to be equally sensitive (Fig. 5) towards initial CMC modification. However, it has been shown that if CMC reactivity is allowed to continue for 24 h the reagent is redistributed as follows¹⁹: 80 CMC molecules mapping between 0.65 and 0.43 map units; 31 CMC molecules mapping between 0.32 and 0.17 map units; 10 CMC molecules mapping between 0.17 and 0.10 map units. This data is based on a low specific activity ^{14}C -CMC probe available at that time that would not detect less than 5 CMC molecules per restriction fragment.

Thus it seems that supercoiling has the capacity to generate well separated localised sites. However, further chemically induced melting seems to coalesce open regions as a function of A-T content²⁷. Although the molecular details need to be resolved for a full understanding of the structural features of DNA that may be varied and generated by supercoiling, it is striking that altered or open secondary structure can be generated at highly preferential sites. This has considerable implications for biological recognition and the modulation of gene expression. Additional evidence supporting the role of supercoiling in transcription has been recently obtained for promoters in plasmids²⁸. Also, some promoters located in linear DNA are sensitive to novobiocin which inhibits DNA gyrase²⁸.

The study of SV40 DNA provides a useful model for the comparative analyses of *in vivo* and *in vitro* functional regions of DNA with regard to the expression and regulation of gene products. SV40 FI DNA can be transcribed by *E. coli* RNA polymerase and the resulting cRNA produces T antigen immunoprecipitated products in a variety of *in vitro* translation systems²⁹⁻³². In addition, linked transcription-translation systems have yielded the major capsid protein VP1, using *E. coli* RNA polymerase³¹⁻³⁴.

Figure 6 is a map of the early and late regions of SV40 DNA showing the sites of *E. coli* RNA polymerase binding. Figure 6a shows that *E. coli* RNA polymerase can initiate at six sites from the early region. These correspond quite well with the mapping

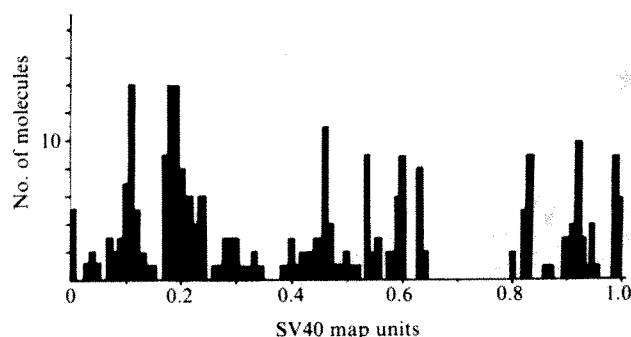
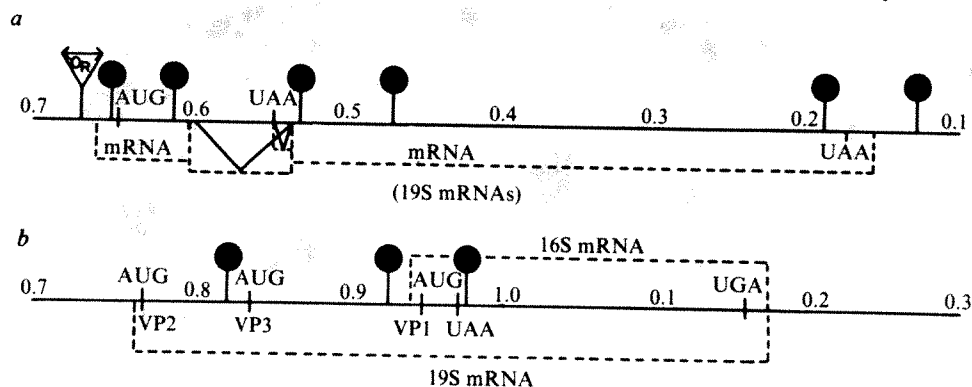


Fig. 5 Map location of *E. coli* RNA polymerase binding sites on 15 min CMC-modified SV40 FI DNA as determined by electron microscopy. Samples were prepared as described in Fig. 1.

Fig. 6 Location of the *E. coli* RNA polymerase molecules in the early (a) and late (b) regions of SV40 DNA. The respective regions of the SV40 DNA map are expressed in a linear form with relative lengths measured from the *EcoRI* position assigned 1.0 or 0.0. In both cases mRNA synthesis is expressed from left to right, 5' to 3'. The origin of replication is indicated by O_R and the cytoplasmic mRNAs are shown by dashed lines. a, The early region contains an intron sequence and two splices occur which are indicated by the large and small $V^{33,45,46}$. The two mRNAs that are produced consist of 0.65 to 0.55 and 0.65 to 0.60 fused respectively to the main body of the message 0.54 to 0.17. The larger mRNA, that is with the smaller spliced out regions, retains the UAA stop codon³³ shown at 0.547 and hence protein synthesis only produced a 17,000 molecular weight (MW) protein, little t antigen. The small mRNA, with the entire intron sequence spliced out produces large T antigen, with an approximate MW of 80,000. The UAA stop codon for this protein is shown at 0.175. b, The late region of SV40 coding for viral proteins VP1, VP2, and VP3. (ref. 33). The lower dashed line represents the 19S mRNA coding for VP2 and VP3 with AUG initiation codons at 0.767 and 0.835, respectively.^{33,34}. These overlapping proteins are terminated by the stop codon UAA shown at 0.969 (ref. 33). The upper dashed line represents 16S mRNA coding for VP1 with initiation of protein synthesis from the AUG codon shown at 0.947 and termination at the UGA codon shown at 0.155 (refs 33, 34). The reading frame for VP1 (16S mRNA) is different than VP2 or VP3 (19S mRNA) and the UAA stop at 0.969 is not recognised³³. The respective AUG initiation and UAA or UGA termination codons have been determined from DNA and protein sequence data³³. No attempt has been made to show the variable 5' leader splicing that occurs from the region 0.721 to 0.760 which is attached to late 19S and 16S mRNAs³⁴. These may be involved in the control of the expression of VP2 and VP3.



of [γ -³²P] ATP initiations at 0.17, 0.45, 0.52, 0.61 and 0.65. The promoter at 0.65 would generate cRNA with translation stop signals³³, hence only the region from 0.65 to 0.55 can be translated. This would produce small t antigen. The promoters at 0.53 and 0.47 would produce cRNAs that could be translated into T antigen-like proteins. The two promoters between 0.20 and 0.10 should not produce any significant T antigen-like proteins. Figure 6b compares the *E. coli* RNA polymerase binding sites of the late region to *in vivo* functions. We observe that the enzyme binds before the appropriate initiation codons that would produce VP3 and VP1. The latter is the major capsid protein whereas VP2 and VP3 represent minor structural proteins^{24,33}.

It is striking that *E. coli* RNA polymerase recognises regions in the correct polarity proceeding AUG initiation codons for two out of three late gene products as well as the AUG initiation codon for T antigens. The other binding sites for the enzyme, except the 0.13 site, all occur before open reading frames with potential codons for initiation. With regard to primary sequence recognition sites, it has already been established that there is a promoter sequence for tight binding of *E. coli* RNA polymerase at 0.165 on the SV40 DNA map³⁵. Examination of the remaining SV40 DNA sequence (ref. 33 and P. T. Chan, unpublished data) reveals promoter sequences³⁶ that could fit with our electron microscopy RNA polymerase binding analysis with the exception of the 0.65 site. However, this site is very A-T rich³³ and may be easily opened to serve as a promoter. Many other potential promoter sequences are also available but not used, suggesting that some of the *E. coli* RNA polymerase recognition sites that are used share similar features with the eukaryotic RNA polymerase II. This is supported by a correspondence of five of six binding sites for the respective enzymes on polyoma DNA using electron microscopy mapping^{37,38}. A recent report indicates that *E. coli* RNA polymerase binds to the appropriate sites on adenovirus DNA for the production of some of the late mRNAs³⁹.

The evidence that supercoiling has a significant role in the modulation of gene expression in prokaryotic systems is quite compelling. The possibility that modulation of supercoiling occurs through the changes in binding of histone and non-histone proteins would be a plausible extension of these results. The finding that superhelical turns makes promoters highly preferential targets for carbodiimide modification opens new possibilities for exploring the structural transitions produced in DNA by the torsional stress of supercoiling.

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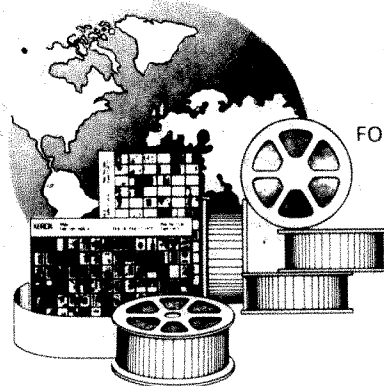
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BOOK REVIEWS

Intelligence for beginners?

Donald D. Dorfman

EYSENCK'S new book on intelligence is his first to appear since Leon Kamin reported gross irregularities in Sir Cyril Burt's data. The main purpose of his book is to present "what to the author appears to be the paradigm towards which the research of the past 80 years converges" (page 6). The book is "intended for beginners" (page 6). An author of a work intended for beginners has an obligation to give a balanced and scrupulous description and analysis of the historical and scientific data.

On page one of his book, Eysenck refers the reader — presumably a beginner — to his appendix A which "gives a short account of the facts of the Burt affair". He implies in that appendix that Arthur Jensen was the first to discover anomalies in Burt's data. Leon Kamin — the actual discoverer — is never mentioned in that historical account.

In his first chapter, "Intelligence: The Development of a Concept", Eysenck concludes that culture-bound intelligence tests are admissible for pupil and student selection at school and university because "we are often justified in assuming considerable uniformity in cultural background among candidates" (page 23). Eysenck gives no justification for his cultural-uniformity hypothesis.

His review of the evidence for a correlation between IQ and amplitude and latency of evoked cortical potentials is misleading. The figures that he displays in support of a strong correlation are taken from Ertl's early work which even Eysenck admits "suffered from technical and methodological deficiencies" (page 50). He then asserts that Shucard and Horn have also obtained "quite sizable correlations between AEP's [averaged evoked potentials] and IQ" (page 50). In fact, those investigators reported a correlation of only +0.24 for fluid intelligence and an absence of correlation for crystallized intelligence in the article cited by Eysenck. He also presents data from "our own laboratories" collected in about 1973. No details are given and no reference is made to any relevant publications in scholarly journals.

Eysenck's introductory text is also interspersed with political comments. For instance, in his discussion of IQ and job success, he tells us that "in our type of society there is little evidence of political

The Structure and Measurement of Intelligence. By Hans J. Eysenck. Pp.253. (Springer: Berlin, Heidelberg and New York, 1979.) DM 44, \$24.20.

and ideological interference with employment and promotion (except perhaps through union activity)" (page 88). In his final chapter "Intelligence and Society", Eysenck reports only one example of abuse of IQ tests for job selection: "it has been shown that on occasion white trade unionists in the U.S.A. have insisted on the use of irrelevant IQ tests for job selection in order to keep out black applicants" (page 221).

"Does IQ Measure Intelligence?" is the title of Eysenck's chapter on the validity of the IQ test. "We shall consider some outstanding studies" (page 79) in this chapter, he writes. One of those "outstanding studies" presented was the investigation performed during World War I by American Army psychologists with the Army Alpha, a group test of intelligence. Eysenck gives some of their evidence for the validity of the intelligence test, for example, their finding that illiterate enlisted men performed more poorly on the Army Alpha than literate enlisted men. Eysenck, however, neglects to present any of the items from that IQ test. Here are a few: (a) "Harvard University is in _____"; (b) "Yale University is at _____". Here are a few more: (a) "Why is tennis good exercise?"; (b) "Lob is a term used in _____"; and (c) "a battle in racket very tennis useful is", which is to be rearranged into a sentence and then answered "true" or "false." The Army Alpha was the first IQ test used to classify national and racial groups according to native intelligence. "The great and universally agreed success of these tests caused many other countries to adopt them in later years and presents another external validation criterion for IQ tests as measures of intelligence" (page 83) Eysenck writes.

The book also contains three chapters jointly authored by Fulker and Eysenck: a chapter on heredity, one on environment and a final chapter on socio-economic status. Their evaluation of the evidence supporting an environmentalist position is quite good overall. Their criticisms of the Heber study are reasonable and their presentation of Zajonc's confluence model

is clear and fair. On the other hand, Fulker and Eysenck's arguments for the genetic position are in no way convincing and often misleading.

In his introductory chapter, Eysenck states (page 1): "I have tried to rewrite the relevant chapters in the history of the intelligence testing movement without including Burt's now doubtful data". Whereas Eysenck may have excluded Burt's questionable data from his historical accounts, Fulker and Eysenck present and make rather extensive use of Burt's fabricated numbers in their biometrical analyses. For example, in their analyses of IQ correlations for monozygotic twins reared apart, they include Burt's group-test correlation of 0.77, asserting that "the main criticism [of Burt's data] concerns the individual test scores" (page 109). That statement is false. Indeed, the main criticism concerns his group-test correlation: it remained constant at 0.771 from 1955 to 1966 in spite of large increases in sample size. Fulker and Eysenck also include Burt's questionable IQ correlations in their analyses of data for siblings reared apart (Table 5.4) and for unrelated children reared in the same home (Table 5.5). In their biometrical analyses of educational achievement, they use Burt's correlations for monozygotic twins reared together, for dizygotic twins reared together and for monozygotic twins reared apart (Table 5.14). Their biometrical exercises lead them to conclude that genotype-environment interaction and genotype-environment co-variance are small enough to be ignored. That conclusion contradicts the results of the statistical exercises presented in their final chapter on socio-economic status. The conclusions are as varied as the models.

Near the end of the book, there is an unnumbered chapter bearing the title "Eysenck and the Splitting of the IQ". A careful study of the primary sources reveals that if the IQ was split, it was split by Furneaux. In fact, Furneaux's first publication on this matter was apparently in *Nature* in 1952 (170, 37-38).

Summing up, Eysenck's new book is inappropriate for beginners, but rather entertaining for experts. □

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Comprehensive palaeontology

J.C.W. Cope

The Encyclopedia of Paleontology. Edited by R.W. Fairbridge and D. Jabolonski. Pp.912. (Dowden, Hutchinson and Ross: New York. Distributed by Academic Press: New York and London, 1979.) \$90, £52.50.

THE appearance of a major reference work on palaeontology within the compass of a single volume is indeed welcome. *The Encyclopedia of Paleontology* contains entries from over 120 palaeontologists, mainly from the USA, but with expertise from Europe, Australasia and Canada contributing some 30% of the information. Most authors have contributed only one entry, others have supplied as many as six.

The subject entries are very wide-ranging and cover in considerable detail each group of plant and animal fossils in alphabetical order. In addition to the taxonomic treatment there is a long list of other subjects covered such as biometrics, computer applications in palaeontology, palaeoecology, population dynamics and so on, in which the state of the art has clear exposition. These two categories of entries are complemented by longer discursive entries on such topics as the history of palaeontology (divided into three separate entries, historically), evolution, extinction and other subjects that lend themselves to such treatment.

The entries are well organized and provide a wealth of accurate information; each has cross-references to other entries and, where appropriate, to entries in other volumes of the *Encyclopedia of Earth Sciences*. The reader is directed to further reading by generally well selected and ample references. Occasionally one feels that the author of an entry has not been sufficiently selective in citation of these; as examples of over-zealous quotation of references "Paleoecology of Inland Aquatic Environments" has 110 references and "Radiolaria" almost 120. This surely is not helpful to the average reader seeking direction to further major references.

The references provide at least a general clue to the date of writing of the entry. Some seem to have been written about ten years ago, for although recent references are listed, no reference is made to them in the text. However, the entries in general are up to date; many articles were clearly written around 1976 and references up to 1978 occur commonly. Within the limits imposed by getting such a volume published this seems very reasonable. Where work is dated, it is largely a reflection of how rapidly some aspects of palaeontology have advanced recently.

On first glancing through the book I

wondered whether it had been mis-bound as there are no entries under 'N' — where were nannoplankton, nautiloids, nekton, neoteny? In fact these fears were largely unjustified as most of the above subjects appear elsewhere under other headings and are listed in the extensive index which, a quick calculation suggests, contains close on 5000 subject entries, many with several page references. The index must be criticized, though, in that it sometimes refers to words which are just mentioned in passing. Thus my search for 'neoteny' led to p.34 which merely stated that certain Cretaceous Amphibia were "pedomorphic or neotenic". The index entry for "pedomorphic" only quotes p.34 so that the reader is still none the wiser on what neoteny is, and both index entries are unnecessary. Checks revealed a number of similar superfluous entries. Misprints in the text have led to index entries; thus "Nautiloids" (*sic*) refers to a misprint on p.533 and is quoted by the index separately from "Nautiloids" and "Nautiloidea". "Conchostraca" get separate indexing from "Conchostracans", but the index entry "Cyclostomata" refers to both the vertebrate and bryozoan orders of that name. Clearly the index was not compiled by a palaeontologist, and its value is consequently somewhat diminished.

Although the book is extremely comprehensive in its treatment, I was surprised that scant attention had been paid to the stratigraphical use of fossils. There is an apposite entry on

biostratigraphy by Peter Sylvester-Bradley (one of seven authors who have since died) — but that is all. There is no entry in the index under the word 'zone', and although some systematic entries do refer to stratigraphical value of particular fossil groups, to me this is a major omission.

The book is abundantly illustrated by both half-tone and line illustrations; the great majority of them are first class and contribute significantly to the volume. The high standard of the majority makes one aware of the number which fall short of this standard in some respects; poor reproduction mars several otherwise good figures, and widely disparate sizes and styles of lettering are distracting.

The price of \$90 (or £52.50) seems excessive for a single volume. At half this price the number of purchasers would probably be increased at least tenfold; but the considerable authority and wealth of information contained therein should compel all palaeontologists seriously to consider buying the book. For undergraduates it will provide valuable information and speedy reference to essential reading for essays and project work; for university libraries at least one copy is essential. Despite its faults this is a volume truly worthy of its title and one which is unlikely to be rivalled for a long time. □

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Kinetics for chemists

C.F. Wells

Chemical Kinetics and Transport. By P.C. Jordan. Pp.340. (Plenum: New York and London, 1979.) £13.55.

As he states in his preface, and is implied in the title, the author adopts a new style in his treatment of kinetics. The first three chapters are concerned with the fundamentals of the transport of molecules: two deal with the kinetic theory of gases, covering the properties of a collection of gaseous molecules at equilibrium and the transport of molecules; but the third introduces an unusual note for a book on kinetics, a treatment of electrolytic conduction and diffusion. In Chapter 4, the experimental approach to kinetics begins with the determination of rate laws. This is followed by a treatment of stationary state mechanisms, photochemistry and non-stationary state mechanisms. The book is then completed by a consideration of single collisions, the theory of reaction rates and various models for collisional processes.

In its concentration on chemical kinetics from a transport viewpoint, especially in the earlier chapters, the book has a novel

approach. Moreover, much important material is reserved for the problems at the end of each chapter; undergraduate students attempting to follow their more conventional lecture courses with the aid of this book might well become confused. The latter state will probably be increased by what some will regard as a cavalier treatment of elementary rate laws. In Chapter 4, complex ideas are introduced too early: for example, before single rate laws are explored, the problems of the $H_2 + I_2$ and the $H_2 + Br_2$ reactions are stated, and we find a discussion of nanosecond flash photolysis; or, before a discussion of simple orders higher than one, we are plunged into opposed first order reactions. Indeed, in a footnote the reader is referred to a standard text to obtain a detailed understanding of the integrated rate laws. Perhaps this is the most unsatisfactory chapter from an undergraduate point of view, as there are also minor contributions to his confusion. For example, in the section on first order reactions, one finds a jumble of first order and opposed first order reactions; or, on p.90, it is stated that the *first* term in eqn 4.31 vanishes, whereas, in the balance of rates at equilibrium, the first *two* terms disappear. However, if the student survives this chapter, or is assisted

in grasping these basic ideas from other sources, he should be able to proceed readily with the rest of the book. It is, perhaps, unfortunate that some of the theories of reaction rates are not introduced earlier than Chapter 9, so that the ideas developed there could be used *en route*.

Finally, I must introduce what is perhaps a more personal note, although I am sure that others also involved with kinetics in

solution will echo my view. Most general books on kinetics concentrate on the gas phase; understandably so, as here individual molecular processes can be examined in detail. Yet rarely are the complexities of processes in solution dismissed so completely: these are merely referred to very briefly in one paragraph in Chapter 5.

Although I have obviously read this book with a critical eye, I realize, of course, that not everything can be included in a

book of this size. Moreover, I am sure that all who are interested in kinetics will find it stimulating, and this includes undergraduates. It certainly should find a place on library shelves. The format of the book is good and it is well produced; the price is not unreasonable for these days. □

C.F. Wells is Reader in Inorganic Chemistry at the University of Birmingham, UK, and has particular interest in the kinetics of inorganic reactions in solution.

Volcanoes revisited

P.E. Baker

Volcanology. By H. Williams and A. R. McBirney. Pp.397. (Freeman, Cooper & Co.: San Francisco; Blackwell Scientific: Oxford, 1979.) £19.50, \$33.50.

THE authors preface their book with the modest statement that it "... does not purport to offer much that is new; it attempts rather, to summarize for the student and professional geologist current knowledge of volcanoes, particularly their behaviour and physical structure". Despite a superficial resemblance to earlier books on the subject of volcanoes (for example, Macdonald, 1972) and some fairly conventional chapter headings, the approach is essentially rather different. It is a textbook aimed at a specialist readership and largely devoid of dramatic accounts of volcanic eruptions and their consequences. It is written in a lucid and cautious style with every effort being made to adopt a quantitative approach. It attempts a rigorous explanation of volcanic processes and products in terms of established physical principles. There are more descriptive chapters, such as those on "Principal kinds of eruptions" and "Cones, domes and shields", which contain innumerable examples drawn from the authors' wide experience.

They pick their way delicately through the subject, rejecting untenable generalizations and sometimes reconsidering discarded theories. They remind us that the evidence for extraordinary amounts of igneous activity along the axes of oceanic ridge crests is largely circumstantial, and that potassium does not invariably increase towards the interior of continents. Perhaps, also, there is some truth in Daly's theory of the 'vitreous substratum' source for basalt.

At the end of the opening chapter the authors state that, "By far the greatest impetus for studies of igneous processes has come from a growing recognition of the central role they play in nearly all modern tectonic theories". Maps of active volcanoes and plate boundaries appear on the inside covers of the book but can scarcely be regarded as reflecting the

dominant theme of the text. There are references to sea-floor spreading, mid-plate volcanism and subduction of oceanic lithosphere, but the authors appear anxious not to overemphasize the link between volcanism and plate tectonics.

The scope of the book has been carefully restricted and there is, for example, very little discussion of the role of volcanism in the evolution of the Earth, or of volcanism and mineralization. Volcanic activity in the rest of the Solar System is also excluded apart from occasional references and photographs of the martian volcano Olympus Mons and a NASA picture of

volcanic activity on Io.

The book is generously illustrated with many first-class photographs and the occasional indifferent one (for example, Fig. 7-11). The figures are drawn in somewhat varied styles but are not always to the standard one might have expected. Nevertheless, in its description and interpretation of volcanic phenomena this is an excellent book and the authors have certainly succeeded in the task they set themselves. □

P. E. Baker is Professor of Geology at the University of Nottingham, UK.

Methods for studying evolution

R.A. Fisher

Biochemical Systematics and Evolution. By A. Ferguson. Pp.194. (Blackie: Glasgow, 1979.) £14.50.

THE popular image of a taxonomist is of a cantankerous anatomist interminably arguing with an equally cantankerous opponent over some apparently trivial detail. These days, it seems, anatomists are out and biochemical geneticists are in. Cantankerousness and argument are, of course, always with us, but perhaps for those who wish to understand rather than stake their reputations, biochemical systematics can throw some new and useful light on the processes of evolution.

Evolution depends on mutations in DNA, and the basic idea behind biochemical taxonomy is to detect the mutations themselves rather than their effects at several removes. Events overtake us with distressing rapidity these days and it is unfortunate for Dr Ferguson in his book *Biochemical Systematics and Evolution* that he missed the opportunity of mentioning the new technique of restriction mapping, since this allows one to examine the actual gene at first hand. But it is neither the easiest nor the cheapest of methods and for some time to come the electrophoretic comparison of proteins will certainly remain the method of convenience, if not of choice. It involves the

investigation of the gene product rather than the gene, but it is much closer to the ideal than the morphometric comparisons of anatomists.

Electrophoresis is a well established and well described technique and much has been published on the interpretation of protein separations and zymograms. It is a pity, therefore, that, having stated that the aim of this book "is to provide the reader with the necessary background to the principles and practice of electrophoresis", the author does not make a better job of it. If this really was his aim, the sections on methods are too brief and in parts misleading. If one were, for example, to try electrophoresis according to the diagram on page 36, one would fail. Electrical contact between the gel and the electrodes is apparently prevented by a plastic film. The chapter which deals with the interpretation of isozyme patterns is again too brief and sometimes inaccurate. In particular, the origins of isozymes could have been more clearly explained.

The second half of the book deals with the interpretation of data, and here Dr Ferguson seems to be on much more familiar territory. He deals concisely and clearly with the various ways of measuring evolutionary distance and the mathematical examples are well explained and easy to follow. It is this part of the book that will really be of use to the audience for which it is intended, "advanced undergraduate and postgraduate students". □

R.A. Fisher is Head of the Comparative Genetics Section in the Nuffield Laboratories of Comparative Medicine at the London Zoo, UK.

Metals and gases

J. M. Thomas

Chemistry of the Metal-Gas Interface. By M.W. Roberts and C.S. McKee. Pp.594. Clarendon Press/Oxford University Press: Oxford, 1979. £26.

MUCH thought has gone into the planning and writing of this commendable book. Where does one begin in the ordering of a topic that is so expansive and multifaceted? Short summaries, however refreshing, tend to distort the truth. Usually they do not faithfully reflect the vast ramifying nature of a complex and important subject. But comprehensive and detailed surveys dull the mind, provoke the thought that "he who increases knowledge increases sorrow" and inevitably tend to bury important signals in oceans of noise.

A book that runs to nearly 600 pages may at first sight appear to belong to the second of these two categories. Not at all. It is so expertly organized and sub-divided into compact but cleanly dove-tailed components that the 13 chapters make for informative and pleasant reading. The first two chapters neatly summarize the basic concepts of the surface chemistry and crystallography of solids. The next two present authoritative accounts of low energy electron diffraction (LEED) for surface characterization, and the various branches of electron spectroscopy (X-ray or UV induced photoelectron spectroscopy, Auger electron spectroscopy) for the characterization of the energy levels of adsorbates and energy levels or bands of adsorbents. In both these topics, the authors, especially Roberts, have made several original contributions. A further two chapters continue on the theme of surface identification and characterization of the adsorbed linkages; one is devoted to the direct spectroscopic approach (infrared and Raman), the other to a short survey of the surface kinetics involved in such processes as molecular beam scattering. There is also a useful account of the kinetic formulations required to rationalize observed rates of adsorption and desorption as measured by, for example, dynamic mass spectrometry.

Following a short illuminating treatment of physical adsorption at 'clean' metal surfaces and the rudiments of surface diffusion, the book devotes four chapters (nearly 200 pages) to gas reactions at the (predominantly) single crystal faces of various transition and refractory metals — for example W(211), Pt(111), Cu(100), and Ni(111). Almost exclusive attention is paid to the behaviour of the diatomic gases O₂, H₂, CO and N₂; metal adsorption on metals is considered; and the particular activity of stepped surfaces (first clearly identified by Somorjai) is reviewed. This section of the book succeeds admirably in placing recent developments in the context

of earlier endeavour, one of the declared aims of the authors. This section is likely to serve as a valuable quarry for all those interested in retrieving critically digested and reliable information of metal interaction with simple gases.

The final chapter on the impact of new experimental developments on heterogeneous catalysis is a little disappointing. Greater emphasis could have been given to the comparability of certain processes, such as the activation of CO and O₂ in homogeneous and heterogeneous systems. The recent work of Muetterties *et al.* (1979) on metal cluster compounds as models for heterogeneous catalysts, and analogous studies by Bradshaw *et al.* (1979) on Ru₃(CO)₁₂, draw attention to the kinship between the exterior of metal clusters on the one hand and exposed metal surfaces — for example Ru(001) — on the other. Similarities in densities of state alone (as exist between tungsten carbide and platinum at their Fermi levels) are not likely to be the sole cause for the observed,

uncanny similarity in catalytic activity of these materials as reported by Levy and Boudart in 1973.

One may point to a few errors of omission: no mention is made of EXAFS nor of the remarkable studies made (by Yagi and others) on reflection electron diffraction/microscopy of clean and contaminated 'elemental' surfaces. EXAFS in particular, with its ability to yield bond distances, coordination numbers and mean square fluctuations of neighbouring atoms, is already making a valuable contribution to our understanding of solid surfaces. But these are very recent advances. The authors had to call a halt somewhere. How many books in this field published less than a decade ago discussed XPS, UPS, AES and several other techniques which are so impressively paraded in this volume? □

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Anion determination

H.M.N.H. Irving

Handbook of Anion Determination. By W.J. Williams. Pp.630. (Butterworths: London, Boston, Sydney and Toronto, 1979.) \$45.

ANALYSTS familiar with Sandell's *Colorimetric Determination of Trace Metals* have long felt the need for an equally authoritative text on anions. Faced with the need to determine a specific anion (and the present book deals with some 75 different ones), we must select one from among the many procedures which may have been published after due consideration of the accuracy and precision required, the length and cost of a determination, whether a few or a large number of samples will be presented, and the apparatus and the operator's skill needed.

The author deals in order with 36 common anions from acetate to vanadate, then with 14 halogen anions, 14 phosphorus oxyanions, and finally 11 sulphur anions. For each anion there is a well referenced review of separation and concentration procedures (which include distillation, liquid-liquid extraction, chromatography and ion-exchange, precipitation, adsorption and special methods as appropriate). Then follows a review of determinations by gravimetric, titrimetric, spectrophotometric and electroanalytical methods emphasizing techniques such as infrared spectroscopy, the use of ion-selective electrodes, atomic absorption and fluorescence, radiometry and miscel-

laneous methods. The reader must usually make his own choice of procedure but in many cases experimental details of selected determinations are given in full. Where reference is made to special apparatus there are excellent line drawings and great pains have been taken to give the structures of the many organic reagents used; their lay-out is, however, inconsistent in that methylene blue is shown as a sulphonium cation on page 550 and as an ammonium salt on page 576, and the formulae for its fluoroborate complex on page 34 is patently incorrect as must be those of the ternary zirconium-calcein blue-F⁻ complex on page 369 and nitron on page 123. Dyes derived from triphenyl methane may sometimes appear with a carbonium ion, a quinonoid structure, or a lactone ring; ionization of a salt is sometimes explicit but the older presentation, for example, CH₃C₆H₄SO NC1Na, is typical of other formulae in which salt formation must be understood.

But this is purely carping at points which will not diminish the great value of this book to practising analysts who have long had to rely on a miscellany of publications for material that is gathered together here in a single comprehensive book. I particularly liked the full and critical discussions of the determination of phosphorus as molybdo- and vanadophosphate, and the determination of halides, alone and in admixture. Despite the price, no analyst or teacher of inorganic chemistry can afford not to have this fine new undertaking within his easy reach. □

H.M.N.H. Irving, formerly Professor of Structural and Inorganic Chemistry at Leeds University, is now Professor of Analytical Science at the University of Cape Town.

24 April 1980

Are books too expensive?

We love books not merely for what's in them. Books feel good, even smell good. Touching them, we rub the grain of their bindings, we skate our fingers across fine paper, we run our thumbs through pages as if they were guitar strings. They feel good whether they are words by Flaubert or encyclopaedias of pharmacology.

In a bookshop, we can rest our arm on a shelf lost in the art of Chinese porcelains as we face a stack of paperback jackets glowing with sex. We may dash into the shop to get out of the rain, and yet walk out with a storm of ideas, provoked by a chapter heading.

Until quite recently, perhaps our only regret, stimulated by an encounter with books in a bookshop, was the certainty that one life could never give us enough time to read all the books worth reading. There once was a time we could pay our bill and walk out into the street with a few titles under our arm, all for the price of the lunch skipped that afternoon.

Today, of course, the prices confound the pleasures of buying books. What we once could purchase for the price of a skipped lunch, now costs us the equivalent of a dinner in a first-class restaurant. And when we get the bill for scholarly books the tally looks like what we might have expected to spend for a meal in a great French restaurant.

Book reviewers for this periodical often complain about the high prices. Young scientists despair at ever being able to afford them. And the rest of us grumble, often attributing the prices to the greed of publishers. What is to be done? Some argue that if publishers of scientific and technical books showed some restraint, restricting the number of symposia or limiting the output of House titles which are of interest only to narrow specialists, then prices would fall. According to this claim the prices of scholarly books are inversely proportional to the population served. Consequently, publishers must choose books for larger audiences.

There are those who suggest that the best way of lowering the prices of scholarly books is to eliminate unnecessary duplication of titles. Take, for example, the book displays at the Federation of American Societies for Experimental Biology meeting in California a week ago, where more than two dozen publishers exhibited their books. Biologists interested in a book on, let us say, interferon, could choose from among five or six new volumes on the subject, each one from a different publisher along the aisle. We would turn fewer trees to pulp and prices would fall if only a single publisher issued a book on each topic, or so this argument goes.

The difficulty with all these solutions is that the regulation of the science book publishing industry would be much like regulating science itself. Scientific literature rides the caboose on the train of science. Where science goes, publishers inevitably follow.

In order to make any of these proposals work, we will require men and women of great vision. They will need, for example, to choose from among the hundreds of symposia held each year those which should be privileged to appear in print. They will need to determine which are destined to be the classics, to be used in classrooms and laboratories over the next decade, and which should end with the after-dinner speech at the closing banquet.

Or consider those books which might never have been published had someone thought them too specialized. Who among us has the power to predict which area of investigation, now at the periphery of science, will some day emerge at the core?

And as for the proliferation of similar titles on the same subject: can any of us pick the author who will write the best book?

It is true that the rain of books falls most heavily on the libraries. With each slash of the budget, librarians can afford to buy fewer books. As libraries, traditionally the principal customers for scholarly publications, purchase still fewer titles, publishers are forced to charge yet higher prices. And so with each twist of the inflationary spiral, libraries and publishers alike struggle to breathe.

Yet neither inflation nor the ineluctable way in which science unfolds offers absolution to the publishers. We all recognize those who maintain their dignity, even under the strain of conflicting market forces, by their good books. There are those publishers, however, who acknowledge that in order to feed their hungry publishing mill, they must grind out lots of books — good or bad, it hardly makes any difference. Some publishers don't even blush when they compare their own books with sausages or shoelaces.

When will it all end? When will we be able to walk into our local bookshop again, pull a volume off the shelf, and pay the bill without anxiety? For the moment the outlook is grim and will remain so at least until the pace of worldwide inflation slackens. Publishers are not immune to the rapid rise in costs experienced nearly everywhere. Paper, binding, typesetting, distribution and advertising costs are likely to scramble to still higher peaks as costs climb generally.

When we learn how to control an economy gone berserk, we may learn how to control the prices of books. □

United States

Threat to marine science lessens

The new draft of the Law of the Sea Treaty has eased potential restrictions on international marine research. But not all nations are entirely happy with the result. **David Dickson** reports from New York

PRESSURE coming largely from the US scientific community has resulted in a number of changes in the proposed Law of the Sea Treaty, under debate in the United Nations since the early 1970s. The changes are aimed at easing potential restrictions on the conduct of scientific research in regions of the oceans directly controlled by coastal states.

Few of those involved in the negotiations are entirely happy with the outcome. Many nations with large off-shore resource deposits — in particular Canada and some of the Latin American states — would have preferred to stay with the strict controls over research initially proposed, while the scientists of other nations would have liked greater movement in the direction of free scientific enquiry.

Both admit, however, that some compromise between the goals of scientific inquiry and the political and commercial demands of resource management has become necessary. And the changes which have been introduced into a new draft of the treaty, approved at the end of a negotiating session in New York earlier this month, will go to Geneva in the summer for what is hoped to be the final meeting before the treaty is agreed and signed early next year.

Earlier drafts proposed giving coastal states almost absolute authority over research carried out in waters under their control had brought strong protests from scientists who claimed that this would be a direct restriction to the freedom of scientific inquiry.

One of the strongest criticisms came from the US National Academy of Sciences, whose ocean policy committee issued a statement in 1977 warning that unless changes were made in the draft text, the proposed treaty would "cripple future marine scientific research" critical to the survival of the oceans and of mankind.

Since then, the treaty's provisions have been modified as negotiators have attempted to tread a delicate path between commercial and scientific interests, the two connected by the fact that information about resource deposits — for example oil and natural gas — can have important commercial implications when licensing or exploration rights are being considered.

At an early stage, for example, the US dropped its demands that one guiding principle should be the "obligations" of nations to provide for the interests of scientific researchers. It has now been accepted that research within a 200-mile

economic zone and on the continental shelf can only be carried out with the consent of the coastal state, but with the understanding that such consent must be granted when the research project is conducted for peaceful purposes and fulfills other specified criteria.

There have also been modifications to the rules governing the publication of research findings. Previously these would have been strictly controlled by the coastal states involved, triggering complaints of potential censorship. Now the conditions under which permission to publish can be refused have been tightly specified, and a researcher must be told of these conditions before his project begins.

Tentative agreement on the three main outstanding issues of interpretation was reached during the New York negotiations, based largely on proposals put to a working committee by its chairman Dr Alexandrov Yankov of Bulgaria, which the US delegation had already indicated it was prepared to support.

Firstly, there was agreement that disputes over the withholding of consent should be referred to compulsory conciliation, where previously there had been no scope for such a decision to be appealed.

There was also agreement that if a coastal state considers that research to which it has given its consent is carried out in a way that violates the treaty, it now has to give the research worker time to amend the procedures, and no longer has the power summarily to terminate the research project.

Finally, the new draft contains revised wording about the conditions under which a coastal state with a wide continental shelf can refuse permission for research in areas of the shelf outside the 200-mile limit otherwise agreed as the limit of economic responsibility.

Here the wording finally agreed was that permission to carry out research could only be refused in situations where detailed exploitation or exploratory operations were already occurring, or were about to occur within a reasonable period of time.

Negotiators in New York spent considerable time over the wording of this paragraph, since ambiguity over the meaning of "detailed" exploration left a number of countries unhappy that, unless test drills were virtually in place, they could not deny scientists access to areas in which commercially valuable deposits might lie.

Indeed, in the final plenary session of the New York meeting the Canadian delegate

argued that the conference might be building "potentially serious interpretation problems" into the proposed treaty with such wording, adding that "my delegation would have much preferred a solution with more specific concrete provisions clearly affirming the rights of coastal states relating to the conduct of marine scientific research on the continental shelf beyond 200 miles."

But US scientists who, with colleagues from the Federal Republic of Germany, have been pushing strongly to ease the initial restrictions, feel that the changes have made the treaty, if not better, at least less unacceptable than previously.

"We still do not feel that the deal on scientific research is as good as it should be," says Dr John Knauss, Dean of Oceanography at the University of Rhode Island. But he adds: "It's now a question of whether we have a treaty with these amendments or no treaty at all". Others feel that, although the scientific community is unlikely to back the treaty actively, it is also unlikely to oppose it in its present form.

Despite agreement in New York on almost 90% of the proposed text for the Final Law of the Sea, some major problems remain before the treaty can be signed. One of the largest is the composition of the council for the International Sea-Bed Authority responsible for allocating mining rights and determining the conditions under which resources can be exploited.

Here the developed countries, keen that the activities of their industries should experience minimal restrictions, are urging that only five of the council's 35 members should be able to prevent a mining contract from being refused — in other words, should have power to grant a contract. (Five of the western developed countries — US, Japan, West Germany, France and the UK — would have seats on the council.)

In contrast, the developing countries are arguing that it would be more democratic for nine votes to be required for a contract to be awarded, and that a similar number would also be sufficient to block the award of a contract, numbers which the western countries feel could throw them in to an uncomfortable alliance with the Soviet-bloc.

Despite such disagreement, UN officials still hope that sufficient progress has been made to conclude the final version of the treaty during the Geneva meeting, and for it to be signed next spring. The next problem will be getting it accepted. And in the US this means convincing the Senate, responsible for ratifying all foreign treaties, that the changes have been sufficient to meet the previously expressed concerns. □

US/UK collaboration proposed for breeder technology study

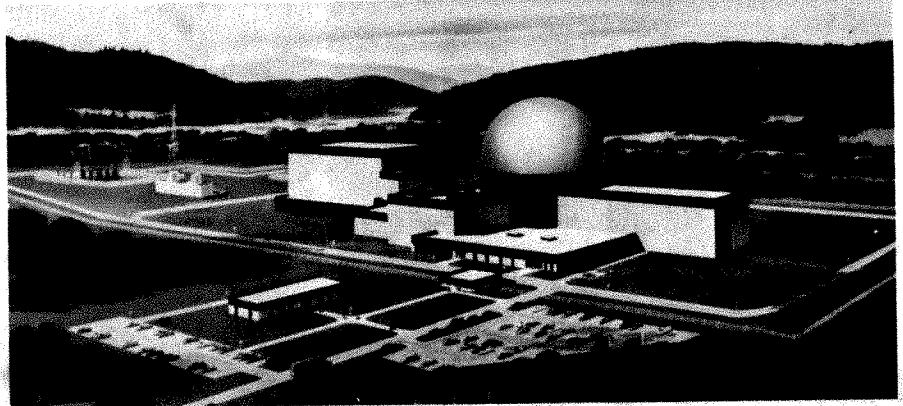
A US congressional committee has approved funds for a joint US/UK study of the future development of liquid metal fast breeder reactor (LMFBR) technology, intended as part of the current programme to build such a reactor at Clinch River in Tennessee.

Precise details on how the money would be spent have yet to be determined. And whether the joint study manages to get off the ground depends largely on how much support can be generated for it in Congress and the administration.

However if the funding is approved the study would, according to US officials, provide a way of formalising the existing interchange of information between nuclear scientists in the two countries, perhaps leading to agreement on efforts to avoid duplication and to help in filling gaps in each other's programmes.

The joint study has been proposed by Energy Production Subcommittee of the House Committee on Science and Technology, which has consistently — and so far successfully — opposed President Carter's attempts since 1977 to terminate the Clinch River project.

Disagreeing with the President that fast breeder development should be delayed either because of the increased risks of nuclear proliferation or because of declining forecasts of energy demands, the subcommittee last year recommended \$183 million be spent in 1980 on the project, which had been dropped entirely from the Department of Energy's budget request.



Clinch River: technical team to cooperate with UK scientists.

Congress subsequently approved spending \$172 million on the project.

This year, after the President had again recommended terminating the project in the budget for 1981, the subcommittee is proposing a budget of \$155 million. And of this, "up to \$15 million" would be for a joint US/UK study with particular emphasis on future fast breeder reactors.

Introducing an amendment to the DOE's budget request, Mrs Marilyn Lloyd Bouquard of Tennessee said that such an effort would be in line with the recommendations made in the final report of the International Nuclear Fuel Cycle Evaluation (INFCE). Ultimately, she said, it would lower the cost of fast breeders.

According to Mrs Bouquard, the proposed joint study would as far as

possible draw upon the existing technical team, design information and data base of the Clinch River project, whose design is virtually finished but for which the DOE has not requested a construction permit.

A spokesman for the United Kingdom Atomic Energy Authority said that already agreement to exchange facilities with the US on fast breeder technology had been reached in September 1979: the UK will subject fuel assemblies to "gross abuses" in the US 'TREAT' test facility, while US researchers will have some access to the prototype fast reactor at Dounreay. But negotiations with the US, and France and Germany, for more extensive exchange on the scale envisaged by the congressional sub-committee were "at a very early stage", pending firm British commitment to a fast reactor policy. □

Panel urges research on tropics before forests disappear

A substantial acceleration of the pace of compiling biological inventories of tropical plants and animals, and a four to five-fold increase in the number of appropriately trained taxonomists to help achieve this, have been recommended in a report on tropical biology by a panel of the National Academy of Sciences. The report, shortly to be published in Washington, warns of the increasingly rapid disappearance of the world's forests.

The panel also lists a number of areas of tropical forests, including the coastal forests of Ecuador, eastern and southern Brazilian Amazonia, and western and southern Cameroon, which it says should receive particular attention by taxonomists over the next 5 to 10 years, since they contain little-known biota in immediate danger of extinction.

And it recommends that a comprehensive laboratory should be established in Puerto Rico as a centre for research and training in tropical physiological plant ecology.

"The creation of such a laboratory appears to be critical for the establishment of a field of investigation that is poorly developed in the tropics. Such investigation appears to offer a great deal

both in scientific and societal rewards" the report says.

The report was prepared by the Committee on Research Priorities in Tropical Biology of the Academy's National Research Council, chaired by Dr Peter H Raven of Missouri Botanical Gardens, St Louis, Missouri.

Speaking in Washington last week, Dr Raven pointed out that less than 10% of the Earth's estimated four to five million species have yet been properly classified, but that while scientific knowledge of tropical ecosystems was virtually non-existent, these areas were being destroyed by population pressures and other demands at an alarming rate.

"I am convinced that 95% of the forests will be converted within 25 to 30 years", said Dr Raven, whose panel predicted that by the close of the century the only extensive areas of undisturbed forest remaining would probably be chiefly those in the western Brazilian Amazonia and in Central Africa. However even those, it says, will probably only persist "for a few more decades".

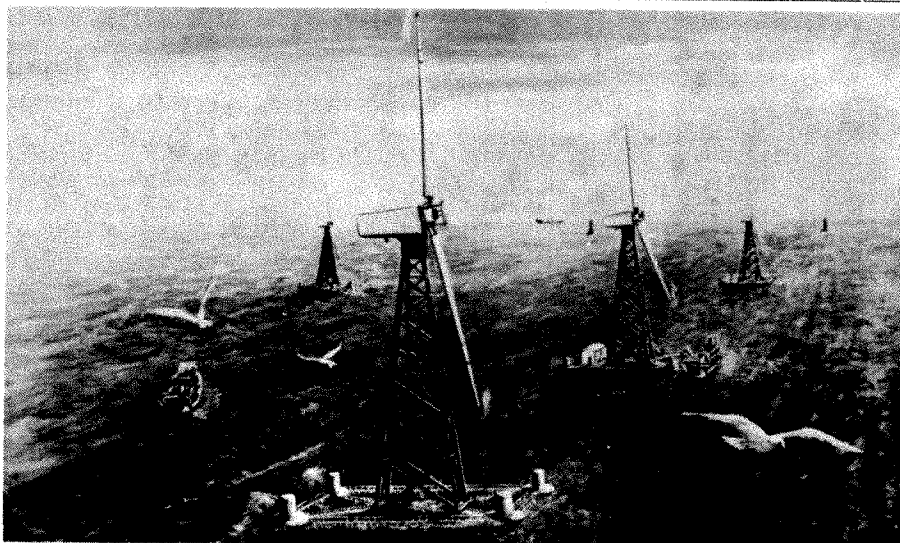
Dr Raven's conclusions were supported by Dr Norman Myers, author of a study *Conservation of Tropical Moist Forests*

commissioned by the panel, which provides a detailed account of the areas currently under threat.

According to Dr Myers, about three quarters of the tropical forests that have already been lost through the actions of millions of forest farmers over the world, who have been able to gain access to the heart of forested areas by using logging trails.

The Academy report warns that if the destruction of the forests continues at the present rate until the next century, the result will be not only widespread human misery and "alterations to the course of evolution", but also the loss of potentially useful knowledge about plant and animal species that might have been used to mitigate some of the consequences of such development.

"This knowledge is therefore not only of theoretical importance, but has critical application of benefit to human welfare both in the tropical countries themselves and throughout the world. If tropical research is allowed to continue at its present level, it will contribute only in a minor way to the solution of the human and scientific problems to which we have alluded" the report says. **David Dickson**



Offshore windmills: can they help conserve fossil fuel?

UK energy

The prospect for ocean-going windmills

THE generation of electricity from windmills sited off the coast of Britain is technically feasible and very likely economic, according to a recent study on the assessment of offshore siting of wind turbine generators commissioned by the UK Department of Energy.

Large arrays of land-based windmills are unattractive in the UK because of the lack of large areas of unpopulated hilly land. However, the UK does have the advantage of a wide continental shelf covered by shallow waters; it also has considerable experience in offshore engineering gained from the exploitation of oil and gas from the North Sea. The study, which should be published in full later this year, has identified several possible sites where the structures for offshore windmills could be built with existing technology. A synopsis of it was presented by P. B. Simpson and D. Lindley of Taywood Engineering — the main contractor for the study — at a meeting of the British Wind Energy Association last week.

Clusters of up to 200 offshore windmills spread over an area of about 60km² could be capable of providing 600MW of electricity to the national grid, the study concludes. Their main economic value would be in the savings they would contribute to the burning of fossil fuels. Machines with 100m diameter turbines placed in areas with an annual mean wind speed of 10m/sec could generate electricity at a cost of 3.4p/kWh — compared to the current cost of about 1.5 p/kWh of generating electricity from a coal fired power station. A similar field of 80m diameter turbines and an annual mean wind speed of 9.5 m/sec could generate electricity at a cost of 5.1 p/kWh.

Unlike conventional power stations,

wind turbines have no fuel costs. The only costs to be factored into calculations of cost per kWh, therefore, are maintenance and capital costs. Moving offshore increases both considerably. According to Simpson and Lindley, the cost of the support structure for one wind turbine (excluding the turbine itself) would be £3 million. One machine would cost £34,000 a year to maintain; and support facilities onshore would have to be built at a cost of £25 million for a 200 machine cluster. Transmission costs for a 600 MWe cluster they estimated to be £171-189/kW — four times that for a conventional 600MWe power station. Construction period and operation time were assumed to be eight and 20 years respectively. They gave no total capital cost for a 200 machine cluster but Dr Peter Musgrove of Reading University, who also participated in the study, believes that a cluster with a generating capacity of up to 1000MWe could be built for roughly the same price of a conventional power station of the same capacity. All costs in the study were at mid-1979 prices.

Most of the capital cost of building wind turbines offshore would be taken up by building support structures which could withstand the rigours of the sea. So the greater the generating capacity of each machine, the more cost effective it would be. Wind turbine diameter is one of the three main factors affecting generating capacity, according to Simpson and Lindley. The other two are mean and rated wind speeds and cluster configuration. Machines spaced at anything less than fifteen turbine diameters are considerably less efficient than individual machines. In the particular sites considered in the study, mean water depth (10-25m), height of

turbine axis above the water (1-1.5×turbine diameter) and distance offshore (5-25 km) had only secondary effects.

These conclusions are based on the detailed analysis of three sites — Burnham Flats in the Wash approaches, Shell Flat off Morecambe Bay and Carmarthen Bay — chosen out of an initial 17 on technical and economic grounds. Lindsay and Simpson stress that these sites are not to be seen as automatic choices for future projects. The study did not include the question of amenity, they say, because it was felt that detailed proposals would be needed first. However, it did assume that all possible sites should be at least 5km from the coast.

If wind power is developed vigorously, thinks Dr Musgrove, it could match the development of nuclear power over the past 25 years and contribute 20% of electricity supply by the year 2000. It should be possible, he says, to build several arrays each containing 200 machines of 100m turbine diameter and capable of generating 5MW each. The net result of each array would be equivalent to one 1000 MW power station operating with a 35% load factor. Variability in wind speed would not be much of a problem because at offshore sites the wind is fairly constant over short periods. The greatest variation is seasonal but this has the advantage that the strongest wind is in winter.

To realise this potential, says Dr Musgrove, the UK should be prepared to support a government funded programme of at least £5 million per year on wind energy research for the next decade, compared to the £½ million spent last year by the Department of Energy; otherwise there is a danger that Britain will fall behind the US, Holland and Germany, its chief competitors in this field. The US and Germany are already building horizontal wind turbines of diameter up to 140m, whereas the UK has not yet received approval to build a 60m diameter horizontal axis machine even though £½ million has been spent on its design.

Dr Musgrove, however, is an advocate of vertical rather than horizontal axis machines. The problem with horizontal axis machines, he says, is that the blades are subjected to gravity loads acting in different directions and this considerably limits their size. A vertical axis machine would not have this problem and could be built to much greater diameters.

So far, however, the UK has gone no further than to design a 25m, 100-150kW vertical axis machine. A decision on further funding for that is also expected later this year. The next six months, therefore, will be decisive for the future of UK wind energy, thinks Dr Musgrove. Not only does a decision have to be taken on what should be done as a result of the offshore study, but decisions have also to be taken on the future of the 60m horizontal and 25m vertical axis designs.

Judy Redfearn

United Kingdom

Science Research Council cuts top grants

BRITAIN'S Science Research Council has been forced to reject £2.2 million worth of "alpha" quality applications for university research, a spokesman said last week.

The Science Board, one of four grant-allocating boards of the SRC (the others are Astronomy Space and Radio, Nuclear Physics and Engineering), meets three times a year to decide allocations. At its first meeting this year the board managed to stave off cuts, except for all "beta" applications — worthy research which "wouldn't disgrace the British taxpayer" but without the star quality of the alphas. Beta research — about a quarter of all applications — has not been funded for the past three years.

But at the Science Board's recent second allocation of the year, it proved impossible to avoid cuts even in the alphas. A total application for £7.1 million was cut back to £4.9 million by applying an across-the-board 30% cut to all of the Board's 10 committees. The committees range from basic biology, maths, physics, and chemistry to science-based archaeology and cognitive science.

The Board spends some £16 million annually on direct research grants to universities, compared to about £3 million each for the astronomy and nuclear boards and £19 million for the engineering board. The cuts thus represent a substantial loss of direct science spending in universities.

"We are most worried about the very few people now going in to new posts in universities" said the spokesman. "We used to be able to be a little generous and allow for the enthusiasm of these 27 to 30 year olds. But there is no more room for the benefit of the doubt. We are cut to the bone."

However, the picture shows some signs of improvement. At the next meeting, the Science Board hopes to have to cut only 15 to 20% of its "alpha" applications, as a result of money beginning to be freed from long term commitments. These are principally the spallation neutron source being built at the Rutherford Laboratory, the synchrotron radiation source at Daresbury, and Britain's one third share of the Institut Laue Langevin at Grenoble. The major part of the spending on these facilities should be over in three years, releasing some £2-3 million a year; and as grants run typically over three years, some of these savings can begin to be accounted now. □



Thaumatococcus . . . sweet discovery

Gene machining sweeter gum

TATE and Lyle, the British sugar manufacturers, are to apply recombinant DNA techniques to improve production of the protein thaumatin — a substance 2,500 times sweeter than a 10% sugar solution.

Thaumatococcus is produced by the berries of the African bush *Thaumatococcus daniellii*. It was discovered in a 1968 survey for new sweeteners conducted by the UK Ministry of Agriculture; and already Tate and Lyle have plantations of the bush in Ghana, Liberia, and Malaysia producing "hundreds of tonnes" of the berries annually.

One tonne of berries produces a kilogramme of thaumatin, which at present is sold only in Japan. Japan accepts thaumatin as a "natural product" which does not require toxicity testing; other

countries require extensive toxicity tests. An application on the basis of preliminary tests by Tate and Lyle has been with the UK Ministry of Agriculture, Fisheries and Foods since January 1978.

Dr John Higginbotham, who is in charge of the thaumatin project at Tate and Lyle's Reading laboratory, estimated future markets as a tonne annually to Japan, with five times that in Europe and ten times in the US. This 150-fold increase could come from increasing the cultivated area of *Thaumatococcus* — but the plant will only propagate in hot climates. The company could avoid "politically uncertain" crops in Third World countries if it could transfer the gene for thaumatin production from the plant to a bacterium, which could then be grown in fermenters anywhere.

Professor Ken Stacey, director of the biological laboratory of the University of Kent, has taken on the task, backed by a £90,000 grant for three years. Professor Stacey thinks three years should see them through to expression of the gene; development of a production process will take longer.

The first task is to isolate the relevant messenger RNA, he says. And ultimately there may be the problem that the bacterial cytoplasm will not encourage the formation of the eight disulphide bonds required in the folded thaumatin molecule. Thaumatococcus has a molecular weight of some 20,000, around three times that of insulin.

The size of the molecule, compared to that of sugar, is one drawback to its wide application as a sugar substitute, said Dr Higginbotham. It is less mobile on the tongue, and so takes "a second or two" to register its sweetness. Lemonade, for example, sweetened with thaumatin, would taste first bitter (from the citric acid) and then sweet (from the thaumatin). Consequently it is only likely to be used in substances like toothpaste, which stay in the mouth for a long time. □

France

Reprocessing plant close to disaster?

THE French nuclear reprocessing plant at Cap de la Hague, near Cherbourg, apparently lost all electrical power on 16 April for an hour, closing down cooling systems on high active waste tanks and ventilation over nitric-acid filled reprocessing vats. Full power was not achieved for another 11 hours.

According to a report in the *Guardian*, a major catastrophe was averted only because the plant was not yet in full production. Auxiliary power was not available because the failure was caused by a fire in a transformer room through which passed both the main and back up circuits. Pierre Tanguy, Head of Safety at the French Atomic Energy Commission (CEA) told *Nature* last week that the back up circuit was not necessarily at fault but "it

would be looked at".

According to Tanguy, there was no radioactive release and no hazard to the workforce or environment. "It was nothing serious" he said. However the French union the Confédération Française Démocratique du Travail have argued in a booklet "Le Dossier Electronucléaire" that a particular danger in reprocessing is "the possibility of cooling caused by the failure of the main and back-up electricity supply". But the accident was not predicted in any official safety studies, says the *Guardian*.

COGEMA, the government-owned company which controls reprocessing in France, have said that the plant would be open again this week.

Robert Walgate

NEWS IN BRIEF

Fusion research boost at Karlsruhe

GERMANY'S nuclear research centre at Karlsruhe, which has for a long period concentrated on fast breeder reactors, has begun to turn its attention to fusion. In its 1980-3 budget Karlsruhe has allowed a 61.5% increase in fusion spending, taking it from 22.6 million DM in 1980 to 36.5 million DM in 1983.

The centre will concentrate on the technology of complete fusion reactors rather than on basic plasma physics, says a report in *Wissenschaft, Wirtschaft, Politik*. It will also construct a superconducting coil for a torus to be built at Oak Ridge National Laboratory in the US, and make some "exploratory studies" of deuterium-tritium inertial confinement.

The greatest effort at Karlsruhe will still be on the fast breeder, but the programme will increase only by 3.5%. The total Karlsruhe budget will increase by 16% from 336 million DM in 1980 to 388 million DM in 1983.

Superconducting technology, on the other hand, will be cut by 5.4%. The main sufferer will be high current superconductivity.

May launch for two Ariane satellites

THE second test flight of the Ariane launcher to place two satellites in orbit will take place between 20-30 May from Kourou, French Guiana, the European Space Agency and the French Centre National d'Etudes Spatiales announced last week. The actual three-hour launch will occur between 1130-1430 hours GMT.

Preparations for the launch, begun on 2 April, are going ahead on schedule following the arrival of the L02 launcher at Kourou. The first, second and third stages of the launcher were erected on 4 April, 8 April and 14 April respectively. The equipment bay was placed in position on 15 April.

The plan of operations has been streamlined compared to the first flight test, L01, thereby reducing the preparation time by about a month. The satellites to be placed in orbit are Firewheel, a scientific satellite designed by the Max Planck Institute, and Oscar 9, a satellite designed by the German Branch of the Radio Amateur Satellite Corporation.

Space shuttle engine test successful

THE National Aeronautics and Space Administration's space shuttle, plagued by a succession of technical problems, received some good news last week when it was reported that the shuttle's engines had

been successfully tested at full power for six minutes. In a static firing test carried out at NASA's National Space Technology Laboratories at Bay St Louis, in Mississippi, the engine operated for a total of 10 minutes and ten seconds — including the first sustained operation at its full 109% of rated power — and met all the test objectives.

Meanwhile the agency is asking private industry to perform a study of thermal protection systems which might be developed as an alternative to the present ceramic tiles which have been giving the shuttle programme severe difficulties and have caused the delay of the first flight to late next year at earliest. The purpose is to find whether any recent technological advances in reusable surface insulation might provide an acceptable alternative. The industry study will evaluate metallic and reinforced carbon compounds.

French commission approves Plogoff reactor

THE three commissioners charged with conducting the nuclear power inquiry in Plogoff recommended last week that the government go ahead with its plans to construct twin nuclear power stations on the scenic Brittany coastline of Cap Sizun. The commissioners based their decision on the observations of 121 people who visited the government's exhibits staged in "town hall annexes" set up after the mayors of the four affected communes, Plogoff, Primelin, Goulien, and Cleden-Cap-Sizun



had refused the government access to their town halls.

The "town hall annexes" had been the subject of militant protest during the 6-week exhibit from 31 January to 14 March, and had to be surrounded by mobile police forces who engaged in battles with the daily demonstrations of up to 20,000 people that were called "la messe" (the Mass) by the protestors. Only 212 of the estimated 60,000 people in the region visited the mobile vans.

French environmental groups attacked the decision, calling the public inquiry a

"grotesque hypocrisy". M. Phillipe Marchand, deputy from Charente-Maritime and Socialist Party spokesman for energy criticised the commission for "ignoring the fact that almost the entire population is opposed to the project and that a number of scientists have denounced the insufficiency and quasi-falsification of the impact studies".

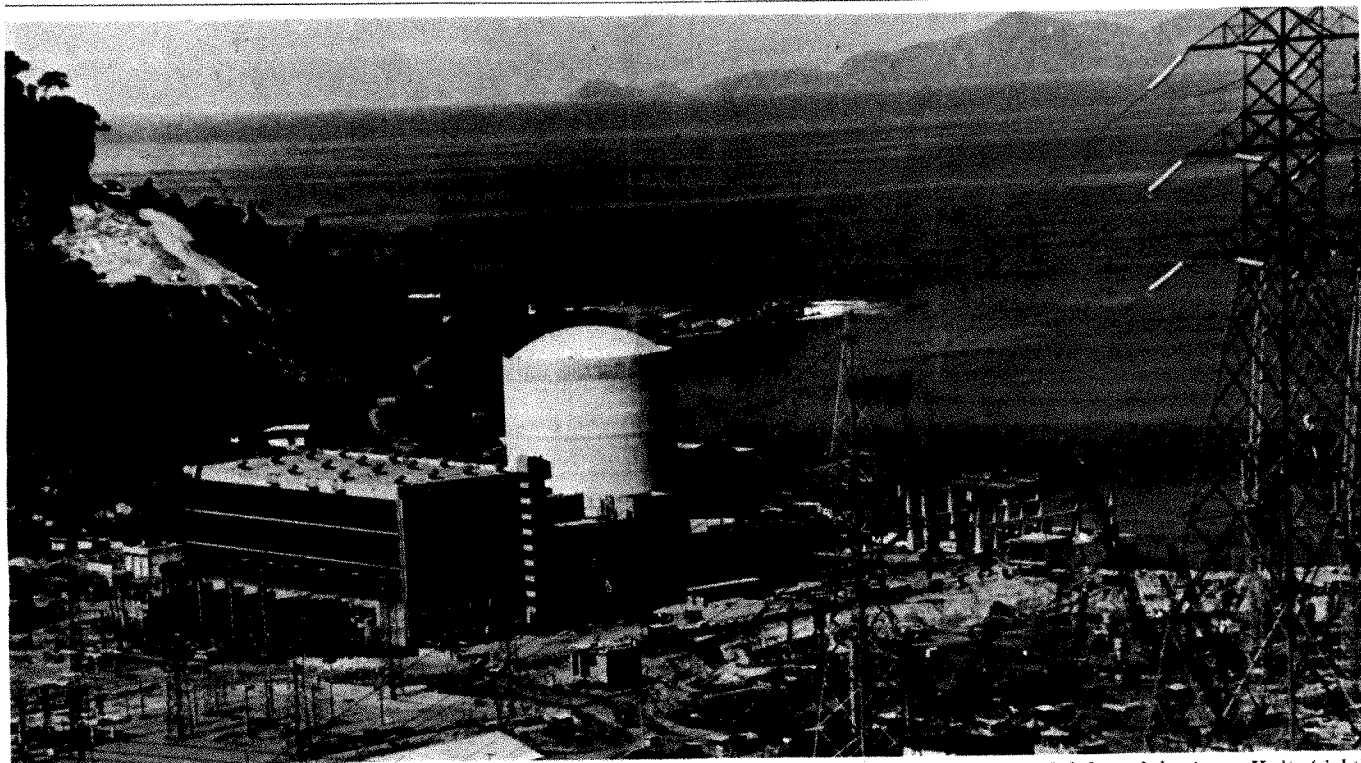
A group of 20 political organizations, trade unions and ecological groups in Finistère, seat of the regional prefecture, denounced "the police over-reaction" during the inquiry and have called for the resignation of the chief of police of Quimper and the dissolution of the prefecture's riot police as "a permanent menace against the right to demonstrate". The French Council of State will take its decision on the reactors after a delay of up to eight months.

Energy conservation cut back

ENERGY conservation spending at the UK Department of the Environment, which is responsible for building regulations, will be £31.1 million for 1980-81, according to a ministerial statement in the House of Commons. This is to be compared with an allocation of £67.6 million in 1979-80, of which £51.6 million was spent. There is no longer a specific allocation for the insulation of housing built by local authorities, said the minister, and grants for assistance with private home insulation have been reduced "to reflect the pattern of demand". According to Gerald Leach's *A low Energy Strategy for the UK*, around 50% of end-use energy is used for low grade heat. A large part of this is used to heat buildings.

British PWR application imminent

A "letter of intent" to build a Westinghouse pressurized water reactor will be issued shortly, the chairman of Britain's Central Electricity Generating Board, Mr Glyn England, said last week. The letter will release "an avalanche" of technical information from Westinghouse, said Mr England. Detailed design of the reactor would then go on within the National Nuclear Corporation, in close consultation with the CEBG and the Nuclear Installations Inspectorate (which will report on safety). A firm application for consent to build would then go to the government at the end of 1981, followed shortly after by the NII safety report. The CEBG hope that the promised public inquiry on the PWR would be held by the spring of 1982, and that if the results are favourable, construction could begin at the end of that year.



On the beach . . . Westinghouse's Angra I (left) and the Angra II site (right)

The politics of power in Brazil

WITH an electricity demand of 25,000 MW and a hydroelectric potential above 200,000 MW — the Brazilian government went ahead in 1975 with a multi-billion dollar nuclear programme. Brazil's nuclear programme is now years behind schedule and in a state of general disarray, as foreign loans are used up and open dissent appears at all levels. **Maurice Bazin** reports

THE original plan, drawn up in the first half of the 1970s, in the heyday of the fanfared Brazilian "economic miracle", called for 60 nuclear power plants, producing 75,000 MW, by the year 2000. It reflected the mood for grandiose projects of that period — the Brazilian version of the American conquest of the West — symbolized by the construction of the Transamazonian Highway. The launching of these projects coincided with a period of harsh military rule and total press censorship. No sobering criticism could be heard and only the promises of the "miracle" were allowed exposure.

Today, the Transamazonian Highway has not brought the expected development. On the contrary, it has only destroyed what was there already. As for the nuclear plan, it has been reduced to eight plants by the year 2000, and there are many calls for a stop to the programme after three plants have been brought into operation, in 1995.

Nuclear energy does not have a good reputation in Brazil. The first attempt to develop a nuclear technology in 1953 ended up in a scandal. The government had convinced three German physicists who had worked for the Nazi nuclear effort during the war, to come and resume their

work in Brazil. Three gas centrifuges for uranium enrichment were to be shipped secretly from West Germany to Brazil. But the shipment was eventually embargoed by the US High Commissioner for Germany, stopping that particular avenue of development.

Until the signing of an agreement with West Germany in 1975, Brazil had remained in the nuclear sphere of influence of the United States. During the Korean war, it delivered monazitic sands to the US Atomic Energy Commission in unlimited quantities and without compensation. Any attempts to develop a national approach on nuclear energy came into conflict with US commercial interests. In the 1960s a group of Brazilian physicists and engineers calling itself the "thorium group" was doing research in Belo Horizonte on the design of a reactor which would make use of the exceptionally abundant reserves of thorium in Brazil. Their recommendations were ignored by government and their research funds cut off after four years, as negotiations for buying a Westinghouse PWR of 626 MW were getting under way. The sale of this first nuclear plant, Angra I, was confirmed in 1972. Construction is nearing completion in Angra dos Reis on

the Atlantic coast, 130 km from Rio de Janeiro. This sale marked the high point of the influence of the United States in Brazilian energy policy.

"The present Brazilian nuclear programme is almost exclusively the result of Brazilian diplomacy and has little to do with national science and technology; those have been relegated effectively to second place, or to a marginal role" wrote Luiz Pinguelli Rosa, secretary of the Brazilian Physical Society. This sour comment reflects the government's total lack of esteem for its own scientists; their suggestion of 15 years ago to develop a thorium-fuelled reactor is now even presented in official speeches as an alternative coming from the US.

The German agreement encompasses all aspects of nuclear technology, from the prospection of fissile materials to the construction of depositories for waste. There would be a whole industry, capable of operating the complete nuclear fuel cycle. But only accessory parts are under construction so far: a fuel rods conditioning plant will be operational this year, and a heavy equipment complex, NUCLEP, for manufacturing boilers, heat exchangers and possibly turbines, is also under construction.

Two parts of the agreement raised most international concern: those for enriching uranium (breaking the American monopoly) and for construction of a fuel recycling plant, capable of extracting plutonium. In its early days, the Carter administration was anxious to stop these two plants. The diplomatic tug of war is

continuing, with the United States trying to restrict the spread of "sensitive" technologies and materials and Brazil insisting on the right to include nuclear energy in its development strategy. In the words of Brazil's chief delegate to the International Nuclear Fuel Cycle Evaluation (INFCE), Ambassador Carlos Augusto Proenca Rosa: "the use of nuclear energy in developing countries was treated by INFCE in a restrictive and limited way; there are insinuations, at times, that the major contribution that developing countries could offer in the field of nuclear energy should be to increase the prospection and extraction of their uranium resource to benefit the importing developed countries . . . We firmly believe that any effective policy of non-proliferation must be non-discriminatory and must bring about measures which are universally applicable, in order not only to guarantee the correct use of nuclear energy by countries which do not possess nuclear weapons, but also to put a stop to the arms race between countries which possess them."

The suspicions repeatedly raised about the Brazilian military government's intentions to develop a bellicose nuclear capacity rely upon past aggression towards Argentina, which led to wars in the 19th century. Today, however, both countries' harsh military regimes are much more interested in signing agreements on exchange of technology, and common ventures, than fomenting regional tensions. Numerous official visits and meetings of the heads of the nuclear programmes of each country have taken place. Each time the peaceful purpose of both countries' nuclear involvement was reaffirmed. Argentina possesses the only nuclear plant operating in Latin America — Atucha I — which functions with natural uranium. It is also negotiating with West Germany's KWU to buy a new nuclear plant similar to the ones being built in Brazil. As the Brazilian programme meets with increasing delays but the industrial capacity for nuclear components develops in both countries, the two countries have entered an era of increasing commercial cooperation, and no-one talks now of any potential enemy to use nuclear weapons against.

But even if Latin America remains free of nuclear weapons, it will not remain free of nuclear waste. To a question about where nuclear waste from the Brazilian programme would be stored, Rex Nazare, acting director of CNEN, the National Commission for Nuclear Energy, responded: "this is not yet defined. But up to six months before the first plant starts operating there is still time to define the location where to deposit waste."

Finding the sites may be difficult, because Brazilian public opinion is becoming aware of the concern that this issue has raised in developed countries. The only site proposed so far is in the mountains

behind Rio de Janeiro, a place with abundant surface water and where a large proportion of the vegetables consumed in Rio are grown. With the modest redemocratization of the military regime an amount of discussion has taken place, which has led the government to abandon the site.

The government presented its justifications for the agreement with Germany as a white paper in 1977. It claimed that nuclear power was "a necessity in view of Brazil's energy needs" and because the price of oil was increasing. It claimed that, between 1940 and 1973, the proportion of imported energy rose from 15 to 40%, and that "the hydroelectric option is approaching its natural economic limit". Paulo Nogueira Batista, director of NUCLEBRAS, the state nuclear corporation, declared that the

German view

● Many of the Brazilian nuclear opposition's calculations on hydropower are "erroneous and foolish", a spokesman for the West German federal ministry for science and technology claimed in a telephone interview last week.

There are no engineers in the opposition group, said the spokesman, and so the costs and difficulty of transporting current from distant hydro stations have been underestimated. Moreover, the Brazilian government had told Germany that by 1995 all available water resources will have been exhausted. "That's why they want to go nuclear."

The contract with Brazil allows for the supply of two reactors, Angra II and III, with options for an additional six. West German participation would decrease in the later reactors, with Brazilian participation rising to 70-90%. "But we would be happy if Brazil approves the Angra III reactor later this year." The deal was important for Germany because "if the nuclear industry wants to be economic they have to produce something", and with nuclear opposition strong in Germany deals with countries such as Brazil and Argentina were attractive. Nuclear opposition in Brazil was decreasing, the spokesman believed.

Pilot plant design for a gas nozzle enrichment plant was nearly complete, and construction has begun on site, but it will proceed at a leisurely pace. For reprocessing used fuel, Brazilian chemists and engineers are in training in Germany and working on the design of a pilot plant.

Safeguards against the diversion of nuclear materials for weapons building are included in a February 1976 agreement between the International Atomic Energy Agency and the Brazilian and West German governments. The safeguards are not "full scope", but apply only to nuclear materials and technology supplied by or derived from West Germany.

Robert Walgate

hydroelectric potential would be used up by 1990.

This technocratic argument was accompanied by reassuring declarations about the safety of nuclear energy. Technical reliability was proven from the "perfect and uninterrupted operation of close to 150 plants in 18 countries with more than 900 reactor-years of commercial service. Thus nuclear energy is the only functional alternative in view of its level of technical confidence and its competitive cost of production." The first two plants to be built with German technology (Angra II and Angra III) were promised for 1982 and 1983 respectively.

In 1974 FURNAS, the electricity supply company, had elaborated a Plan 1990 for electrical energy needs. The data presented formed the basis for the technical justifications of the agreement. It posed a growth rate of the demand for electrical energy of 11.4% per year, linked with the growth of GNP. It estimated an investment cost per plant of \$500/kW and claimed a load factor of 80% for nuclear plants, while the hydroelectric load factor in Brazil is only 50%, due to seasonal variations in rainfall.

In 1979, however, FURNAS presented a revised Plan 1992, which estimated a demand growth rate of only 7.5% a year. Considering that Plan 1990 itself had stated that "nuclear plant participation would be reduced to zero" for a growth rate below 8.7%, the revised figure becomes equivalent to proposing abandoning the whole programme. It revised the investment cost up to \$1,700/kW. The load factor of nuclear plants was brought down to 65.5% based on plants operating in the West.

During the intervening five years Brazil's "economic miracle" had come to a brutal halt: GNP grew only by 4.1% in 1977, compared to 11.8% in 1975. As censorship slackened, various sectors of the Brazilian technocracy started questioning publicly the economics of the agreement. In 1979, General Dirceu Coutinho, who headed NUCLEI, the subsidiary of NUCLEBRAS which will produce isotopes, resigned and denounced the expense of the programme.

Although criticisms of the programme had been voiced by scientists as early as 1975 at the annual meeting of SBPC, the Brazilian Society for the Progress of Science, and by the Brazilian Society for the Progress of Science, and by Brazilian scientists in exile, the defection of those locally referred to as "nucleocrats" has occurred only during the past two years. As the National Congress started functioning again, it set up a Commission of Inquiry to investigate the programme. David Simon, who had headed the Angra I project and was advisor for nuclear affairs to the president of FURNAS, resigned and collaborated with the Congressional Commission, presenting detailed testimony as a technical expert. He wrote: "apart from a reduced minority of experts — mainly to be found in the world of nucleocrats — there exists a quasi unanimity in the scientific

and technical community around a set of criticisms to be made of the nuclear agreement between Brazil and Germany . . . With the present difference in cost between nuclear and hydroelectric plants, and the availability of hydroelectric sites to the end of this century, there is no need to install nuclear plants before 1990." Indeed, Professor Jose Goldemberg, president of the Brazilian Physical Society, points out that hydroelectric reserves had been purposely underestimated by a factor of two (at 100,000 MW) by FURNAS's Plan 1990.

David Simon makes a detailed cost comparison between the Angra II and III projects and an equivalent 3,400 MW hydroelectric plant. While Angra I and II together will cost \$5,000 million, the hydroelectric plant would cost \$2,700 million. The fuel costs for the nuclear plants during their lifetime would be an additional \$4,000 million (water is free).

On the purely economic side, the agreement specifies that half the costs will be paid by Brazil in Deutschmarks, while hydroelectric technology would be almost entirely costed in Brazilian currency.

His testimony also pointed out that the jet-nozzle technique for uranium enrichment to be used in Brazil is still under development in West Germany (with half the costs for research and development borne by Brazil) and that its technical and economic viability have not been proved.

It is difficult to imagine the possibility of a reconciliation between the government representatives and the scientific community over the nuclear programme. Paulo Nogueira Batista, president of NUCLEBRAS, and Said Farhat, Minister of Information, called a joint press conference last month at which Nogueira discarded FURNAS' revised Plan 1992, because "the 7.5% growth rate demand envisaged in the plan is perilously below a reliable value", and Farhat emphasized "the Brazilian decision to follow through with the international agreements linked to the execution of the nuclear programme. Being a decision of the government, this is no more subject to either divergences or dissensions. To build the nuclear plants we shall spend US \$15 billion. For the nuclear fuel cycle, we estimate an investment of US \$2.5 billion."

On the other side, Professor Luiz Pinguelli Rosa, Secretary of the Brazilian Physical Society, told *Nature* that the scientific community today demands "democratic and public discussions of energy needs, involving various sectors of the population. We, as scientists, should not fall into the temptation to propose a progressive solution as a substitute for the technocrats' miracle. The illiterate majority of the Brazilian population must first obtain the means to evaluate what nuclear energy is. Before it really needs nuclear reactors, I hope that Brazil can reach the stage of a democracy with popular participation." □



Angra II columns . . . not enough

Trouble on the beach of rotting stone

ANGRA I is a standard PWR reactor of the type sold by Westinghouse all over the world under turn-key contract. Its construction made no use of the national manufacturing capacity: the share of local suppliers in the project was only 8%, made up of civil engineering works.

Start up is promised for next year. It uses enriched uranium provided exclusively by the US, paid for via a loan from the Industrial Development Corporation of South Africa. Used fuel will be shipped back to the US, so the electrical company which will operate Angra I will not

comment on wastes on fuel reprocessing.

The doubling of the construction time is blamed on the agreement with West Germany to build two plants (Angra II and III) on the same site. Besides the obvious dangers involved in having three nuclear plants on one beach, there were other problems. While the water table was being lowered to construct the foundations, the already existing buildings slid 10 millimeters from their position. It was decided to build an "armoured diaphragm wall" around the site, 250 meters long and 17 meters deep. As work proceeded on the anchoring of foundation columns for the reactor platform, it was discovered that the ground below the beach was scattered with huge boulders which had to be pierced to reach bed-rock at a depth of 60 meters. This difficulty could have been anticipated had the engineers recalled the difficulties encountered while building the road to Angra, when a tunnel collapsed to form a huge open trench, through which the road now passes; or had they recalled that the beach's name, "Itaorna", comes from Tupi, the language most spoken in Brazil 250 years ago, meaning "rotting stone".

Setbacks also affected the construction of Angra II. Last year, the National Commission on Nuclear Energy, CNEN, which is responsible for safety, decided that the 280 columns already in the ground were not strong enough against earthquakes. As a result, the construction of the reactor base plate was halted for a year, and CNEN ordered an additional 88 columns and reinforcement of 202 of the already existing ones.

It has been pointed out that CNEN's director has always been considered pro-American, while NUCLEBRAS' is pro-German; stronger rifts could exist between them than soil vibrations.

The overall financial charges from these modifications and delays amount to \$320 million, mainly in the form of interest payments. All these costs will be borne by the electrical company FURNAS alone; it has already announced that it will raise its prices by 60% when Angra I comes on-line next year. There are clauses in the German contracts which pass the cost of technical difficulties onto the Brazilian user company; these clauses remain secret and a Sao Paulo newspaper was seized last year for publishing one of them.

The financing of the German programme is the largest loan ever obtained for a Brazilian project: it amounts to \$1,700 million, and with costs tripled from the original predictions, money is getting short. No electrical company is willing to take on the responsibility for the construction of any further plant beyond Angra III. The government and NUCLEBRAS are pressuring CESP, the utilities company of Sao Paulo, to build a fourth plant (the third of the German agreement), but CESP prefers to build more hydroelectric plants at a quarter of the cost of nuclear plants. □

CORRESPONDENCE

Swiss vote did not decide disposal issue

SIR,—In the issue of 27 March, page 294, under the heading "Power dissenters through Europe", was a report on an anti-test drilling vote in the Swiss village of Högendorf. The assertion that this vote "may have blocked effectively further nuclear development in Switzerland" is unfounded and should be corrected.

The cooperative Nagra has been charged by the Swiss government and the utilities with nuclear power plants, with working out a national disposal concept by 1985, and test drillings are an integral part of the project plans. Högendorf is one of 12 sites in north-east Switzerland for which drilling applications will soon be submitted.

Because nuclear waste disposal is recognized to be a national issue, it was clearly specified in the revised nuclear law accepted by the Swiss public in 1979 that the government is directly responsible for decisions on disposal questions. This responsibility includes any preparatory investigatory work such as the proposed test drillings.

The vote in Högendorf was of a consultative nature. This implies that the government will take such indications of local opinion into account as far as possible, but must also consider the broader, national issues involved. While the applications are being considered project work on waste disposal is continuing on a broad front including planning of further drilling in geological formations other than the deep crystalline rocks currently being investigated.

Yours faithfully,

H. ISSLER
C. McCOMBIE

Nationale Genossenschaft für die Lagerung
Radioaktiver Abfälle, Baden, Switzerland

UK science budget: now the bad news

SIR,—Your report (3 April, page 391) that the science budget will be relatively stable for the next four years misses one important point. Less than half the funds of the Agricultural Research Council comes from the science budget; the majority comes from the Ministry of Agriculture, Fisheries and Food. This has been cut for 1980/1 by an average of 6% but some research institutes will, at constant value, have lost 15% of their 1979/80 budget level by 1981/2. There is no promise of stability for this funding and our fear is that it will decrease further.

I am not out, at least on this occasion, to indulge in special pleading on behalf of agricultural research, or for research in general. It is important, however, that you and your readers are aware that for at least one of the research councils the outlook is neither as good nor as stable as you implied.

Yours faithfully,

JOHN GOODIER

Agricultural Research Council, London

Genothetically speaking

SIR,—The use of new techniques in molecular biology has allowed the fragmentation of the genome of an organism and the introduction of its pieces into adequate hosts where the entire genome can be stored for the desired use when required. This collection of genes is termed in the laboratory jargon and also in the specialized literature "a library of genes". Library is defined in several English dictionaries both British and American as "a place set apart to contain books for reading,

study or reference; a collection of books, especially one for use by the public". Therefore it seems that the expression "library of genes" is inadequate. (This fact becomes more obvious taking into account that library is specifically derived from the Latin word for book, *liber*.)

The discovery of new phenomena or the introduction of new concepts may need the introduction of new words to characterize them precisely. I feel that in the case mentioned above the creation of a new word is fully justified. I would like to propose the word *genotheque* to designate the collection of genes of an organism introduced into an appropriate host species. This short word is derived from *gene* and the greek root *θηκη* (box). This last root appears with the same meaning as that proposed in several English scientific words (perithecium, spermatheca) and in other languages in the word for library (biblioteca, bibliotheque, Bibliothek.)

If we have discoltheque, why not *genotheque*?

Yours faithfully,

CARLOS GANCEDO

Instituto de Enzimologia del CSIC
Facultad de Medicina. Universidad
Autónoma, Madrid-34, Spain

Ke Xue not pukka

SIR,—In his letter (3 April, page 394) Gerard Piel disputed with my statement in a recent *Nature* article that "There are no cover-to-cover translations into Chinese" of foreign scientific journals as yet, by pointing to *Ke Xue* which is the Chinese edition of *Scientific American* being circulated in China. It needs however, to be clarified that this magazine is not regarded by the Chinese as belonging to the category of primary journals, which was the object under examination in my article. Another reason why I did not mention *Ke Xue* was that its publication is a joint exercise and not a solely Chinese effort.

Yours faithfully,

T. B. TANG

Darwin College, Cambridge

Wallis's biblical gesture

SIR,—In Kings Norton's obituary (20 March, page 288) of Sir Barnes Wallis, he mentions that Wallis presented his £10,000 award for his wartime dambusting bomb to a charitable foundation (to help educate the sons and daughters of men who died serving with the RAF). Why he did this deserves to be known. In the book *The Dam Busters*, Paul Brickhill relates the tremendous effort expended for the German dams' raid. When it was radioed by 617 squadron that the Mohne and Eder dams were breached, Sir Wallis was ecstatic that the huge work succeeded. Afterwards, though, he learned that 50% of the attacking planes were lost with the death of 53 very young men. He was shocked and saddened, and said "Oh, if I'd only known, I'd never have started this!" Many years later he was presented the above award. When Paul Brickhill asked him why he gave it away, he replied: "My dear chap, go and read your Bible, turn up Samuel II, Chapter 23. You probably haven't a Bible, so I'll tell you this story about David.

"He was hiding in the cave of Adullam after the Philistines had seized Bethlehem, and in his anguish he said, 'O that one would give me a drink of water of the well of Bethlehem, which is by the gate!' Now the three mighty men who were his lieutenants were with him, and I'm dashed if they didn't fight their way through the Philistine lines and draw a goatskin of water out of the well by the gate.



They fought their way back and took the water to David in the cave, but when they told him how they had got it, he would not drink it. They asked him why, and he said: 'Is not this the blood of the men that went in jeopardy of their lives?'"

Yours faithfully,

P. D. MORLEY

University of Texas, Austin, US

Optical effects in taxis

SIR,—I wonder if any of your readers can explain a strange optical perception effect I've noticed when travelling in a London taxi-cab? No-one I've described it to has a simple explanation; and I assure you it is not restricted to trips taken after alcoholic lunches. The effect is most visible at night.

London taxis, like many others, have a vertical glass partition between the passenger seats, which face forwards, and the driver. Behind the passenger is the rear window of the cab, through which can be seen the receding road, street lights, other vehicles and buildings.

The glass partition acts like a partial mirror; in it one sees a reflection of the view through the rear window, and through it the back of the driver, the meter, and part of the view through the front cab window.

The effect — and when one has spotted it it is quite marked — is that the objects reflected in the partition glass (ie those in the view through the rear window) appear to be receding very fast, much faster than the cab is apparently travelling judging by the view forward through the partition. Subjectively, the impression is of about twice the cab's real speed. I first noticed it with the reflection of traffic lights receding at night as the cab passed them.

The effect disappears completely if one turns around and looks directly out of the rear window; there one's perception is roughly as expected. Turning back to the reflection of the same view, the recession seems to speed up again.

The only explanation I can think of is that the fixed angular size of most of the through-screen images (the back of the driver, the cab) and the increasing angular size of others (the road forward) provides a reference frame against which the decreasing angular size of the reflection is correctly perceived. Turning around, the frame of the window is too peripheral to provide an effective reference, and the brain's mechanisms for maintaining a constant perception of size of an object despite its distance come into play. Is this a possibility?

Yours faithfully,

ROBERT WALGATE

20, Roy Road, Northwood, Middlesex

NEWS AND VIEWS

Pores for thought

from Peter Newmark

MEMBRANE pores, like teeth, can be probed, filled or extracted. Each approach can be useful in the investigation of pore structure and function and each has its advocates. In time the evidence obtained by different approaches should merge but to judge by a recent meeting*, this has not happened yet.

The sodium and the potassium channels that generate action potentials in nerves continue to provoke a good deal of interest and controversy more than a quarter of a century after Hodgkin and Huxley put their electrophysiology on a mathematical basis. The Hodgkin-Huxley model proposed that each channel had a gate which was normally closed but could be opened by depolarisation of the nerve membrane. They postulated that the gates contained three charged particles, and that their successive movement in response to depolarisation allowed ions to start flowing through the channels. It was predicted that their movement should generate a detectable gating current preceding — and much smaller than — the ionic current. The detection of gating currents became a reality in 1972 when both C. Armstrong and R. Keynes recorded them from the sodium channel of the squid giant axon. Keynes (University of Cambridge) now believes that his original conclusion that the gating currents associated with the activation of sodium channels fitted the Hodgkin-Huxley three particle model is wrong. He now gives a mathematical description of the time course of the changes in gating current in terms of two exponential functions and a single constant. This equation is not compatible with the three particle model but nor does it lend itself to any other obvious physical model. Armstrong (University of Pennsylvania) meanwhile obtains what appear to be somewhat different gating currents from the same preparation and describes them with a far more complex equation than that of Keynes. But, like Keynes, Armstrong concludes that the simple Hodgkin-Huxley model is an inadequate physical explanation of gating currents. His tentative model is considerably more complex and also

without an obvious physical counterpart.

The problem of finding physical counterparts to electrophysiological data is that of how to relate an alteration in electric field to a movement of the gating particles — in biochemical terms a conformational change — which brings about the opening of an ion channel. Also to be explained is how, for example, a low pH acting from the inside, and only from the inside, of frog muscle almost completely prevents the closing of sodium channels without having any effect on their opening and the peak sodium flow through them. (W. Nonner, University of Washington, Seattle — see *Nature* **284**, 360; 1980).

Biochemists, at least, believe that all will be revealed once they have purified the protein that encompasses the channel in question. For that reason Y. Ovchinnikov (Shemyakin Institute, Moscow) set a considerable workforce to the task of purifying the sodium channel protein from the nerve in the muscle of the walking legs of 35,000 crabs. The protein thereby isolated has an overall molecular weight of about 250,000 and consists of four subunits of which a 76,000 molecular weight component associated with a 56,000 molecular weight component is the target for a photoaffinity labelled scorpion toxin. However, W. Catterall (University of Washington, Seattle) has a different photoaffinity-labelled scorpion toxin which picks out a much larger protein (250,000 molecular weight) from either neuroblastoma cells or rat brain synaptosomes together with a 32,000 molecular weight protein from the latter. These proteins are shown to be associated with the sodium channel both because mutant neuroblastoma cells that lack sodium channels also lack the 250,000 molecular weight protein and because either depolarisation or unlabelled toxin are capable of blocking the photoaffinity labelling of synaptosomes. (*Proc. natn. Acad. Sci. U.S.A.* **77**, 639; 1979).

The biochemistry of calcium ATPase is far more advanced. Of its 1000 amino acid residues 830 are already known (N. M. Green, National Institute for Medical Research, Mill Hill) and from that and other evidence it is possible to infer how the protein is threaded in and out of the sarcoplasmic reticulum membrane in which it sits. Perhaps with the complete

sequence it will be possible to see whether the calcium channel is formed between dimers or through a monomer. Intuitively, Green favours the monomer theory as does J. Kyte (University of California, San Diego) who argued cogently for a reappraisal of the current dimer theory of channel formation for sodium, potassium ATPase.

The acetylcholine receptor is another protein of interest to both biochemists and electrophysiologists. After two molecules of acetylcholine have bound to their receptor, a water-filled pore opens allowing ions to pass through. Purification of the protein yields a major 40,000 molecular weight protein and several minor components whose roles seem to be becoming less clear. In particular J.P. Changeux (Pasteur Institute) reported that the minor component which he had previously thought to be the 'ionophore' could not be involved in ion flow because it could be removed by alkaline extraction without loss of activity.

The function of two other minor subunits is also unclear and attention is now firmly focused on the 40,000 molecular weight major subunit. Its amino acid sequence, derived either from the purified protein or from DNA, reverse transcribed from messenger RNA, should be available shortly.

Meanwhile the goal of consistently and convincingly reconstituting purified acetylcholine receptor in lipid bilayers whilst retaining ion flux and binding parameters continues to elude P. Mueller (Eastern Pennsylvania Psychiatric Institute) and many others. Changeux, however, claimed some success in reconstituting into small lipid vesicles the acetylcholine receptor of alkaline-treated, solubilized synaptic membranes of the Torpedo electric organ. The key to success, he claimed, was the addition of exogenous lipid.

One good reason for wanting purified reconstituted acetylcholine receptors is that it will enable more sophisticated electrophysiology to be carried out. But even with *in situ* receptors, great advances have been made with the advent of single channel recording. This technique, pioneered by E. Neher and B. Sakmann of the Max-Planck-Institute for Biophysical

*An International Titisee Conference on Membrane Channels held at Titisee, March 6-9 1980, sponsored by Firma Dr. Karl Thomae GmbH.

Chemistry, Goettingen has recently been applied in that Institute to desensitized channels of extra-synaptic membranes of denervated frog muscle. The desensitized form of the channels is an inactive state adopted about 10 sec after the channel has converted from closed to open form in the presence of high concentrations of acetylcholine. Sakmann reported that desensitized channels can suddenly become active again, rapidly fluctuating between open and closed states for less than a second before returning to inactivity. After a very brief delay the cycle may be repeated; after several repeats, there is a prolonged period of inactivity before bursts of activity reappear.

Neher reported the considerable technical achievement of extending single channel recording to potassium channels of the squid axon, although not under normal physiological conditions. This is the first time that the opening and closing of a single channel in a biological membrane, other than those induced by acetylcholine, has been recorded. Currents of 1.7 to 7.0 pA and a mean opening time of 12 msec were recorded. More interesting was the fact that short interruptions could be seen during the open time recording, indicating a temporarily closed condition between the closed and open states. On average there were 1.8 interruptions per open channel recording. According to Neher this refutes the Hodgkin and Huxley model which, for potassium channels,

would predict 0.4 interruptions per channel.

Single channel recording is becoming an extremely important technique for confirming and extending what has been learnt from gating currents and fluctuation analysis in the last few years. It is technically demanding particularly in terms of obtaining sufficiently good seals between the recording pipette and the tissue membrane. But there is every reason to believe that the first recordings of single sodium channels will be reported soon and will be followed by those of channels induced by a variety of neurotransmitters.

An utterly different approach to determining single channel currents, but one applicable only to the much studied model of gramicidin A channels induced in lipid bilayers, was reported by D. Urry (University of Alabama Medical Centre). By means of sodium-23 nuclear magnetic resonance he was able to demonstrate two sodium binding sites and to derive the relevant rate constants. From them and using a model based in part upon the X-ray diffraction data of Koppe *et al.* (*Nature* 279, 723; 1979) Urry then calculated a single channel current which agreed well with observed values. The validity of this approach having been proven to Urry's satisfaction, he expects soon to determine the actual positions of the binding sites within the channel. That will perhaps conclude the exhaustive studies, exhaustingly reported, of the exact way in which sodium ions make their way through the gramicidin channel. □

Peter Newmark is acting editor of *Nature*.

Climatic perturbations

from A. Henderson-Sellers

REINFORCEMENT of climatic perturbations by the natural feedback mechanisms in the atmosphere was a predominant theme of the First International School of Climatology*.

Probably the most exciting indications of internal feedback response amplifying natural climatic variations were the results presented by J. Namias (Scripps Institution) and W. Broecker (Lamont Doherty Observatory). Namias' theories are the culmination of many years of scientific investigations of synoptic meteorology. He presented examples from the USA of atmospheric reinforcement of anomalous situations in various conditions from arid to arctic. (There is an interesting analogy here with the Northern European Drought of 1975/76 which, it has since been suggested, may have persisted because of the extreme conditions of low soil moisture and high reflectivity which it had itself produced). Perhaps more important for those interested in the onset

of ice ages was Namias' further observation that baroclinic development in arctic regions seems to lead to the 'steering' of depression systems along the snow/ice boundary. This gives rise to a positive feedback mechanism in which any precipitation which occurs will 'feed' the snow advance.

The second novel case of an ecosystem response tending to enhance climatic perturbations was described by Broecker. Recent results suggest that the level of carbon dioxide in the Earth's atmosphere was considerably lower (~200 p.p.m.) during glacial epochs compared with the present day (320 p.p.m.). This decreased level of CO₂ would, of course, tend to lower average surface temperatures by decreasing the 'greenhouse effect'. However, the lowered CO₂ level may itself be a result of the onset of continental glaciations. Broecker believes that when sea levels fell as the ice mass on the

Northern Hemisphere continents increased there may have been a sudden input of phosphorus to the oceans due to leaching from the then dry continental shelves. The increased phosphorus levels would stimulate increased photosynthetic activity in the oceanic biomass and thus lead to lowered levels of CO₂ in the atmosphere. This mechanism provides a link between Northern Hemisphere glaciations and Southern Hemisphere temperatures and also indicates the importance of internal responses within the biosphere and hydrosphere for the enhancement of climatic anomalies. It does not, however, explain the origin of the decreased temperatures that triggered the original continental glaciations.

The extreme importance for climatic change of the oceans and particularly the biomass within them was re-emphasized by J. Woods (Southampton) and B. Bolin (Stockholm). Woods presented new and exciting data which indicate that there is a large-scale, but previously neglected, interchange of both water and heat between the major world oceans. Bolin's work on the carbon dioxide budget also underlines the desperate need for a fuller investigation of the oceanic contribution to global sources and sinks of CO₂. Bolin and J. Smagorinski (Princeton) both noted a 4-year fluctuation in the observed levels of atmospheric CO₂ which becomes apparent when the annual oscillation forced by industrial output and biospheric uptake is removed. There is a strong possibility that the atmospheric CO₂ levels are responding to increased uptake by the oceanic biomass during periods of enhanced upwelling. This is another link between atmosphere, climate, sea surface temperatures and the biosphere. One is tempted to reconsider these complex interrelationships in the light of Lovelock's 'Gaia Hypothesis' (Oxford University Press, 1979).

In view of the extremely complex nature of the response of the biosphere to climatic changes it was reassuring to hear from H. Fritts (Arizona) that, given a large enough sample size, biological data (especially from tree rings) could be shown to provide satisfactory reconstructions of past climates. The elegance and strength of the mathematical techniques used in this type of reconstruction (which now allows Fritts to draw surface pressure charts for earlier epochs) may lead to biological data superseding the traditional historical data sources for climatic reconstructions. Although the response of trees to climatic variation may be complex, the signal is, at least, not further confused by intentional modification of the true situation (as is known to have occurred in certain mediaeval manorial reports of crop yields written for absent landlords). Climatic reconstruction and monitoring thus cannot be successfully developed without detailed consideration of both anthropogenic and environmental systems with which the atmosphere is intimately linked. □

*A NATO Advanced Study Institute, held at Erice, Sicily on 9-21 March, 1980. The proceedings will be published by Reidel.

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ON the premise that the climate has changed in the past and thus may well change in the future a Government 'think tank' — the Interdepartmental Group on Climatology — was set up to 'consider whether the United Kingdom was putting sufficient effort into studying the climate'. This group, under the chairmanship of Sir Kenneth Berrill has just delivered its first report*.

The review of the present state of knowledge on climatic change (with special reference to the United Kingdom) is clear, succinct and realistic, but restricted to short-term changes. Major emphasis is given to the carbon dioxide issue (fossil fuel burning and deforestation leading to increased atmospheric carbon dioxide leading to possible global warming), and interesting estimates are given of the economic effects of a 1°C warming (a saving of £250 million per year through reduced energy demand), and the cost of a single severe winter (in excess of £80 million). This section ends with the rather optimistic statement that "within a few years sufficient progress will be made" in predicting the effects of global climatic change on the United Kingdom that climate trends will be able to be considered in future Government planning. This statement implies an understanding of the causes of natural and anthropogenic climatic change which we do not have at present, and would be viewed with scepticism in some circles.

As a review of the present state of knowledge, the Report is deficient in its neglect of studies of climatic change which are based on historical or proxy

*Climatic change. Its potential effects on the United Kingdom and the implications for research, London, Her Majesty's Stationery Office, 1980.

Climatic change

from T.M.L. Wigley

data. This is disappointing since a number of world authorities in these areas are British, and British scientists have been involved in some important recent works. For example, the verification that orbital changes have a major controlling role in determining the cycle of ice ages (Hayes *et al.*, *Science* 194, 1121; 1976) is believed by many to be the major work on climatic change this decade. It might be argued that long-term climatic change (the topic of the Hays *et al.*, paper) was not of any immediate relevance. However, analysis of the past few hundred thousand years has revealed evidence that very rapid climatic changes have occurred in the past (see, for example, Hollin *Nature* 283, 629, 1980). Such changes, if they occurred today, would undoubtedly have a dramatic impact on society. The Report's emphasis on the instrumental record and computer modelling tends to reflect the work on climatic change which is being conducted in the U.K. Meteorological Office. As such it creates an unbalanced impression of work in progress in the U.K. as a whole. This impression is reinforced by the summary of research funding. Only £1 million per year is expended directly in support of research on climatic change: divided into studies of past climates (£¼ million per year) and climate modelling (£¾ million per year). From other figures quoted in the Report, it is apparent that the bulk of this expenditure is within the Meteorological Office. But how good are these estimates and how well do they reflect the work on climatic change which

is being carried out in the United Kingdom?

If one were to consider all research into past climates which is being conducted in the United Kingdom, then the figure of £¼ million per year would be an underestimate. However, a significant amount of funding in this area comes from outside the U.K., and it seems that the Report has considered only U.K.-funded research. Since this restriction has not been stated, the figures tend to be misleading — and a more complete analysis would be of great interest. But how can one properly review all U.K. research into the many facets of climatic change? Funding figures can be deceptive, even if correctly assessed, since certain items of research (such as climate modelling) are extremely expensive. How many scientists are working in the area, how much material is published, and how are these figures distributed amongst the various government bodies, the universities and private industry? These are important questions which the Group has not yet considered.

It is a pity that this Report is restricted to a review of research within, or funded by, the Government. By so doing, a considerable amount of research which is important in the study of climatic change has been neglected. Unfortunately, except in the title no statement is made of the restricted nature of the Report, and, because of this, it can only be misleading to the uninitiated. The Interdepartmental Group on Climatology has, however, not been disbanded, and we can, perhaps, look forward to a more comprehensive review at a later stage. □

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Sign language and spoken language

from James E. Cutting

WHAT is the nature of language? This question has taken on new vitality with our increasing recognition of a whole family of languages previously ignored by linguists, philosophers, psychologists, and neurologists. These languages are the sign languages of the world used by communities of the deaf in enclaves scattered about the globe. These languages are, we suspect, as varied and different as are spoken languages; they are, we now recognize, not at all parasitic on the spoken languages around them; and they have been, regrettably, little studied so far.

How do sign languages differ from spoken languages? How are they similar? What is characteristic of language in general, encompassing both signed and spoken languages? These were among some of the fundamental questions which were discussed at a recent conference*.

By comparison with spoken languages, little is known about sign languages and the only substantial study to date is that of Klima and Bellugi (*The signs of language* Harvard Univ. Press, 1979) on American sign language (ASL). Cross-linguistic study with Chinese Sign Language, French Sign Language (FSL), British Sign Language (BSL), and others has only just begun. One of the interesting facts about these languages is that users of ASL find FSL much more intelligible than BSL, undoubtedly because the American and French sign languages are historically related to one another and quite independent of BSL. This is important

*A Dahlem workshop on Sign Language and Spoken Language, held in Berlin 24-28 March 1980, under the auspices of Senat der Stadt Berlin and Stifterverband für die Deutsche Wissenschaft. The proceedings will be published later this year by Dahlem Konferenzen, edited by U. Bellugi and M. Studdert-Kennedy.

evidence for the independence of the signed from the spoken languages that surround them.

At first glance one might suspect that sign and speech are quite different, if for no other reason than we have two hands for articulation but only one tongue. Interestingly, however, in sign language bilateral gestures in which either opposition or symmetry between movements of the two arms is used seem to be rare. Historical analysis suggests that the evolution of signs may be in the direction of simple one-handed forms.

We can consider sign to entail the shape and movement of the dominant hand (signers can be right- or left-handed) from one place of articulation to another with coordinate gestures of the head and shoulders — just as speech may be thought of as movement of the tongue from one place of articulation to another with co-ordinate closures and releases of the lips and larynx. The places of articulation for signs are centered in two distinct areas. One is



100 years ago

Seeing by Electricity

It may be well to intimate that complete means for seeing by telegraphy have been known for some time by scientific men. The following plan was suggested to us some three years ago by a picture in *Punch*. Our transmitter at A consisted of a large square made up of very small separate squares of selenium. One end of each piece was connected by an insulated wire with the distant place, B, and the other end of each piece connected with the ground. The object whose image was to be sent by telegraph was illuminated very strongly, and, by means of a lens, a very large image thrown on the surface of the transmitter. Now it is well known that if each little piece

of selenium forms part of a circuit in which there is a constant electromotive force the current passing through each piece will depend on its illumination. Hence the strength of electric current in each telegraph line would depend on the illumination of its extremity. Our receiver at the distant place, B, was, in our original plan, a collection of magnetic needles, the position of each of which was controlled by the electric current passing through the particular telegraph wire with which it was connected. Each magnet, by its movement, closed or opened an aperture through which light passed to illuminate the back of a small square of frosted glass. There were, of course, as many of these illuminated squares at B as of selenium squares at A, and it is quite evident that since the illumination of each square depends on the strength of the current in its circuit, and this current depends on the illumination of the selenium at the other end of the wire, the image of a distant object would in this way be transmitted as a mosaic by electricity.

From *Nature* 21 April 22, 589; 1880

around the face, generally centred between the eyebrows and mouth and off-center to the cheek ipsilateral with the dominant hand and the other is in front of the signer with the nondominant hand often used as an articulator surface for the dominant hand to play against. The coordinate gestures of the head and shoulders, however, play a different part in sign from the part played by lips and larynx in speech. Head and shoulder movement seems to serve, in part, grammatical purposes, such as denoting relative clauses, and they play no role at a sublexical level. The lips, on the other hand, play no grammatical role in any known spoken language, but are intimately involved in sublexical units.

Lexical items in speech are composed of phonemes. English has about 50. Lexical items in sign are composed of formational parameters — handshape, place of articulation, movement, and orientation — that yield somewhat more than 50 cheremes (Stokoe et al, *A dictionary of American Sign Language on linguistic principles*, Gallaudet College Press, Washington, 1965). These parameters have a somewhat different status in sign than phonemes, or even syllables, in speech; yet slips of the hand are often parameter-based just as slips of the tongue are often based on phonemic and syllabic structure. Virtually all hand configurations can go with any movement, with virtually any place of articulation, yielding as many as 10,000 possible ASL signs.

The interesting point is that these parameters are arrayed simultaneously, whereas the phonemes and syllables of speech are strewn out in time. It may be that the difference in modality has encouraged differences in manner of composition of linguistic units. Visual display may allow a more simultaneous

arrangement, and auditory display a more sequential one. Sign languages, particularly ASL, appear to use this simultaneous, spatial character in many ways.

For example, morphological functions can be overlaid on lexical items without necessarily increasing the amount of time taken to produce the sign. This is done by modulating the movement parameter of the sign such that both the original movement and the modulation can be seen. Consider the ASL sign for BLACK, articulated in citation form with the forefinger of the dominant hand extended as the hand sweeps across the eyebrows from the contralateral to ipsilateral side of the face. To change this to VERY BLACK the sweep is made with an accelerated beginning; to change it to BECOME BLACK the sweep is made with an initial tenseness or hesitation; to change it to HABITUALLY BLACK it is made repeatedly; and so forth. In this manner the lexicon of the language is syntactically enriched through the use of morphological additions quite unlike those used in English. Moreover, these operations are performed on the base sign in an essentially simultaneous layering. Spoken languages seem to be rather impoverished in terms of this kind of layering; the best candidates seem to be the vowel harmonies of languages like Finnish, the insertion of different vowels into consonant groups according to grammatical function in Hebrew, and the use of tone for grammatical purpose in certain dialects of Chinese.

A second example of the use of space by sign that has simultaneous character may be found in anaphora and pronominal reference. In sign discourse, the signer "lays out" in space for the sign recipient the actors and objects that are referred to. This is done laterally, left to right or vice versa, indicating a single place for each person or object to occupy, using as many

as four, five, or more locations. These locations are set up as they occur in discourse, and both signer and sign recipient remember their places. In this manner, pronouns analogous to *she* or *it* are actually locations in front of the signer, set up arbitrarily in discourse and held there simultaneously as long as is convenient, and removed when necessary. Spoken languages seem to have no analogue to this, partly because acoustic space may not allow tone heights to be used pronominally. It would simply be clumsy and confusing.

Thus, simultaneous layering of linguistic units and simultaneous display of anaphoric relations may be ways in which sign languages have been encouraged by modality to structure themselves. Yet simultaneity of display in vision may have costs in sequentiality. Consider proposition rates. Semanticists have used the notion of a proposition as a unit of discourse. For example, *John is a secretary* is a sentence with one proposition, and *John is a tall secretary* is a sentence with two, roughly corresponding to *John is a secretary* and *The secretary is tall*. There are many schemes for counting propositions, and semanticists have long debated which way is the best, but regardless of the scheme, sign languages (at least ASL) seem to convey only about two thirds of the propositions per unit time that spoken languages do. Thus, it may be that the visual modality encourages simultaneous layering precisely because it is constrained sequentially.

Sign languages force a broadening of our notions of what language is. Indeed, we are often surprised by what we find in sign. Surely more surprises are in store. For example, we currently have little idea of what difference there may be between apraxia and aphasia of the deaf who have suffered cerebral impairment. Yet these are useful diagnostic categories for the hearing in clinical settings. In addition, we have no real idea why second-generation signers are better than first-generation signers — that is, their language is richer — when in fact the second-generation users learned their language, by and large, from first-generation users. Answers to these and other questions will come. What will also come with increasing inquiry into sign, and this is surely the best byproduct of all, is an increasing recognition and liberation of the deaf. Traditionally, deaf peoples have had to learn in oralist mode, one particularly ill-suited to their handicap. At best they have been tutored using a language form still far removed from native sign. In the United States this form is called Signed English, which takes the lexical items of ASL and strings them together according to English syntax. Even in this situation deaf signers have been overtly discouraged from using their native language. Increasing inquiry into the sign languages of the world by deaf and hearing researchers can only help the deaf to realize their full potential. □

How long is the charmed lifetime?

from Graham Shaw

By remarkable techniques akin to the systematic location of needles in haystacks, experimental physicists have made considerable progress towards determining the lifetimes of charmed particles. In so doing, they have confirmed yet another fundamental feature of the unified theory of weak and electromagnetic interactions (see *Nature*, 282, 131; 1979).

According to theory, the lightest charmed particles should decay by weak interactions, since charm itself is conserved by the more powerful strong and electromagnetic interactions. And these weak interactions should be the same as those responsible for familiar processes like muon decay, or nuclear beta decay, with precisely the same coupling constant. However, because the charmed quarks are so much heavier than the strange quarks to which they decay, the density of allowed final states available (the 'phase space') is much greater in charm decays than in, for example, muon decays, so that the predicted lifetimes are much shorter. How much shorter depends on the charmed quark mass, but with reasonable values, lifetimes τ in the range 10^{-12} to 10^{-14} s are obtained, compared to 2.10^{-6} s for the muon lifetime.

Turning to experiment, such short lifetimes $\tau \sim 10^{-13}$ s present a serious challenge, since in a typical charm production experiment they imply decay lengths of a few hundred microns. This distance is of the same order as the bubble size in a typical bubble chamber, so that the resolu-

tion required is on the edge of what may be achieved with this technique. Very much better spatial resolution can be obtained by observing charmed particle production and decay in photographic emulsion stacks. In this case, the problem is not to resolve the details of a given event, but to find it. The events are relatively rare, and in general occur deep in the stack, so that it would take innumerable man years of effort to locate them in sufficient numbers to be interesting. A method is required to locate the needles in the haystack, without sifting every straw. This is provided in a series of so-called hybrid experiments by placing an array of electronic counters and/or a bubble chamber behind the stack (see *Nature* 279, 287; 1979). These can be used to detect the particles produced together with the charmed particle, and the decay products of the said particle, when they emerge from the stack. They serve a triple purpose: to select out possible charmed particle events from more numerous backgrounds; to identify and measure the momenta of the emerging particles; and by tracing back the flight paths of the particle into the stack, to locate the small region in which the event occurred.

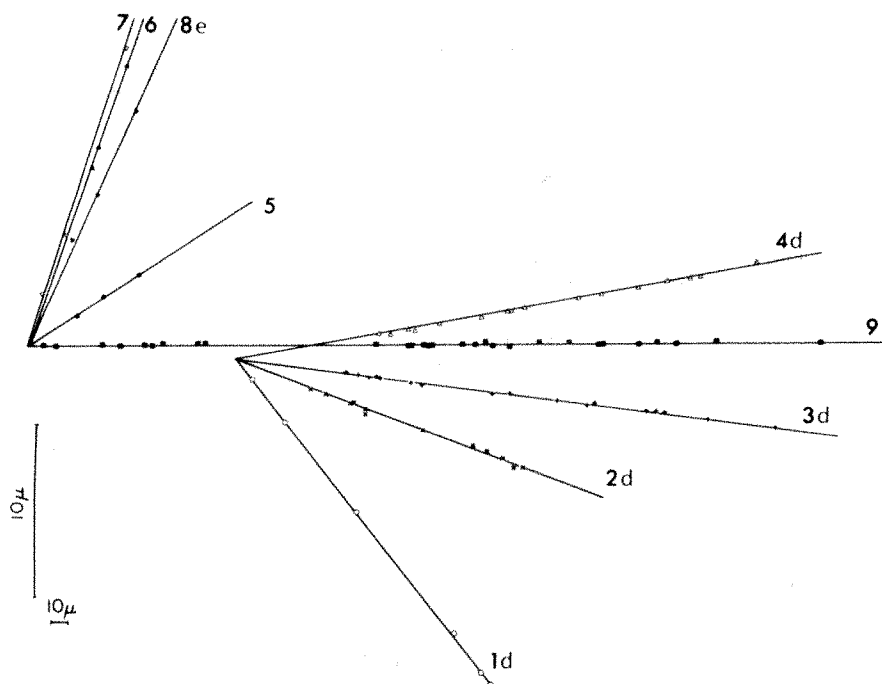
An example of an event located in this way is shown in the figure, with best fit lines drawn through the measured positions of grains in the emulsion (Adamovich *et al.*, *Phys. Letters* 89B, 427; 1980). In this experiment, an 80 GeV tagged photon beam is incident from the left, and the event clearly indicates a neutral particle being produced at the first vertex, and decaying at the second. Information from the external counters indicates $\pi^+\pi^-\pi^-K^+$ as

the most likely identification for the decay products 1d, 2d, 3d, 4d, and leads to a mass for the decaying particle of 1866 ± 8 MeV/c², coincident with the mass of a charmed \bar{D}^0 meson. On the basis of this and other detailed considerations, the authors conclude that the production and decay of a \bar{D}^0 meson has almost certainly been observed. The distance between the production and decay vertices is measured in the emulsion as $122.7 \pm 2.2 \mu\text{m}$, with a corresponding decay time of $(2.26 \pm 0.05) \cdot 10^{-14}$ s.

Similar events have also been seen in several other hybrid experiments (see Voydovic, *Proceedings of the International Symposium on Lepton and Photon Interactions at High Energies*, Fermilab, Batavia, in press). In particular, a group working at Fermilab with a neutrino beam, has reported as many as ten events in which the decaying charmed particle can be identified (Prentice, *ibid.*). These include two Λ_c baryon decays with a mean decay time of about $4 \cdot 10^{-13}$ s, four D^\pm or F^\pm meson decays with a mean decay time just under 10^{-12} s, and four D^0 meson with an appreciably shorter mean decay time of about $7 \cdot 10^{-14}$ s.

These experiments, and others like them (Voydovic, *ibid.*) clearly show that charmed lifetimes lie in the expected range, providing further striking evidence for the universal coupling of the weak interactions. They also begin to reveal more detailed features, like the relative shortness of the D^0 lifetime, which has aroused considerable theoretical interest (see Rosen, *Phys. Rev. Letters* 44, 4; 1980; Bander *et al.*, *ibid.* 44, 7; 1980). Hopefully, this is only the beginning. □

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Dendrochronology

from Sara Champion

CURRENT European research in dendrochronology is extending the possibility of accurate dating back into the 1st millennium B.C., and in doing so is confirming observations which could previously only have been made by the conventional method of artefact cross-dating.

When Bruno Huber, the founder of European dendrochronology, died in 1969, his master chronology for Central European oak stretched back from the present for over 1000 years (Huber, *Handbuch der Mikroskopie in der Technik* 5, 171; 1970). He had shown that the considerable variations in the ring widths of coniferous trees in North America caused by extreme temperatures, which allowed the crossmatching of skeleton plots, did not occur in the tree population in the more temperate European climate, rendering this method of crossmatching inapplicable. Concentrating on oak, the

most commonly used building timber and one suitable for interregional study, he developed the use of the agreement percentage and the fractional disagreement percentage for the evaluation of similarity of different plots. His use of the semi-logarithmic scale making the curves independent of the magnitude of absolute growth facilitated the visual comparison of different sequences. His emphasis on the recognition of *indicators* and *signatures* resulted in the establishment of several important ones: the peak in A.D. 1421, the minima in A.D. 1311, 1320 and 1326, and in particular the A.D. 1530-1540 'saw', a sequence of five alternate rises and falls which has been matched in Hollstein's West German oak chronology and has been recognised even in Becker's silver fir sequence.

Recent work has concentrated on improving the procedure for the synchronisation of individual curves, (Eckstein & Bauch *Fortwissenschaftliche Centralblatt* 88, 230; 1969; Baillie & Pilcher *Tree Ring Bulletin* 33, 7; 1973) and on extending the existing chronologies both geographically and temporally.

Chronological sequences have been constructed using the widths of the rings as a basis of comparison. For periods where sequencing of growth rings yield inadequate information for cross-matching, it has been suggested that the density of the wood or the dimensions of the cells may more clearly differentiate the years. The use of X-ray densitometry for establishing the proportion of low and high density wood (Fletcher and Hughes, *Bull. of the Fac. of Forestry*, Univ. of British Columbia 7, 41; 1970; Hughes and Sardinha, *J. Microsc.* 104/1, 91; 1975), has particular significance for dendroclimatology, since it yields detailed information on climatic conditions, but also serves to distinguish individual growth rings more clearly than simple ring width. Density is affected, however, not only by climate, but also by soil type, position of the sample in the tree, and by species so much further research is required if density is to be used alongside ring width for establishing chronologies. Variations in the dimensions of wood cells from year to year also yield detailed climatological information, and may in the future be suitable for use in constructing chronologies.

The identification of a felling year is straightforward if the sample still retains all the sapwood and bark, but research is showing that the approximate felling year can be calculated if the transition between heartwood and sapwood is present, even if most of the latter has been removed because it rots more quickly. Researchers have produced different estimates however, for the number of years to be

allowed between transition and final ring. Recent work on the timespan between felling and construction, based on the examination of timbers for drying fissures, has shown that generally wood used in buildings, bridges and other structures was not seasoned before use (as opposed to wood for cabinet-making, panels etc.), and that construction year can be assumed to be within a year of felling, apart from obvious cases of re-used old timber.

Many centres in Europe have concentrated on constructing regional chronologies, and on extending them back into early history and prehistory. Hollstein's work at Trier (summarised in *Brit. Arch. Reports* 551, 33; 1978) has aimed at establishing a chronology valid for West Germany, Luxembourg and parts of France and Switzerland. This master sequence is closely comparable to Huber's sequence (the 1530-1540 'saw' has been recognised in over 100 cases), but stretches back to A.D. 822 without serious problems. Although there are gaps in the 8th and 4th centuries A.D., use of historical dates such as the construction of the bridge over the Rhine at Cologne in A.D. 310 has allowed Hollstein to extend his chronology back to the 8th century B.C. Differences in elevation, soil and climate between Central and Western Germany and the North German lowlands have necessitated the construction of local sequences, many of which run from the 10th century A.D. to the present. Two chronologies for Sweden extend back to the 6th and 9th centuries A.D., one for Denmark to the 14th (with an earlier section 694-1191), and one for Schleswig-Holstein to the 5th century A.D.

In the Netherlands Bauch (*Brit. Arch. Reports* 551, 133; 1978) and others have established two non-matching chronologies for oak. Their chronology I (continuous back to 1385 A.D., earlier section 1036-1325 A.D.) can be synchronised with the Hollstein and Huber sequences, but their chronology II (1109-1637±1 A.D.), derived from panel paintings and sculptures executed in the Amsterdam and Leiden regions, bears little relation to these mainland European sequences. It has recently been matched, however, with the Type A chronology established for South-East England, with a high agreement percentage for the period 1354-1588 A.D. The import of timber from England is unlikely and there are, anyway, differences in the quality of the panels so the similarities in the curves have been attributed to like patterns of slow growth. A problem remains in that no examples of the Netherlands chronology II curves have been found apart from the panels; it is suggested therefore that the timber came from a source completely exhausted by 1650, the date from which Dutch panels always show the ring pattern of chronology I.

In England, the two main types of chronology identified, A and H, can be

related to continental sequences. Type A, found in South-East England and Yorkshire, has strong similarities to the Netherlands chronology II, while Type H, found in the West and on higher ground, can be correlated with the Huber and Hollstein sequences. Fletcher (*Brit. Arch. Reports* 551, 154; 1978) suggests that there may be a connection between the two chronology types and the two species of oak, sessile and pedunculate, and their preferred environment; current research on species identification should illuminate this problem.

Species other than oak are under investigation. The silver fir has been shown not only to share signatures with oak (the 1530-1540 'saw', for example), but also to be uniform in its growth pattern over a large area from Czechoslovakia to the Vosges. Hollstein's extension of the secure 1100-year fir chronology back to the 7th century B.C. through finds at the Magdalenenberg, a tumulus in the Black Forest, has enhanced the potential of this species. A chronology for beech runs back to the 14th century A.D. in Germany; research continues on the more problematical series for spruce, pine, ash and larch.

The significance of this work for archaeology is crucial. More than 300 dated samples from the important Viking trading centre at Haithabu, Schleswig (Eckstein and Liese, *Germania* 49, 155; 1971) have allowed a detailed constructional history of the site to be made: building dates for houses, wells and pavements span a period of 118 years, and evidence for sequence of structures and re-use of timbers has been produced which would have been unobtainable stratigraphically.

Uncertainties over the correct calibration of radiocarbon dates and variability in measurement have resulted in an extreme reluctance to use this dating technique in Europe for sites of the 1st millennium B.C. Dendrochronology is now producing absolute dates for sites previously dated by relative methods. Excavations at the Magdalenenberg produced timber from the primary and several secondary graves. Hollstein dated the primary grave to 577 B.C., and the secondaries to between that date and 550 B.C.; on archaeological grounds the graves would have been dated to 600-550 B.C. A rich grave at Altrier, Luxemburg, dated archaeologically to 475-400 B.C., produced a *terminus post quem* of 473 B.C. for a piece of timber with no heartwood/sapwood transition present, and a felling date of 461 B.C. for a piece of charcoal found with the cremation.

These dendrochronological dates have confirmed those suggested on archaeological grounds; once the many floating chronologies for material of the 2nd, 3rd and 4th millennia B.C., currently 'dated' by radiocarbon, are fitted to the master curves, the earlier phases of European prehistory may become dateable in calendar years, causing perhaps some upsets to established archaeological sequences. □

SPRING BOOKS SUPPLEMENT

This above all...

G.S. Stent

THIS is the second of a series of books commissioned by the Alfred P. Sloan Foundation in which according to its President "a representative selection of accomplished and articulate scientists [are] to set down their own accounts of their lives in science", in order to further the public understanding of science. Who could be more fitting for such a commission than Sir Peter Medawar, supreme among contemporary scientists for his combination of scientific excellence, erudition and literary skill? But rather than simply composing another scientific autobiography, Medawar has "tried to write the kind of book I myself should have liked to have read when I began research before most of my readers were born": a young scientist's vade-mecum that offers succour and savvy to the innocent, as provided by an experienced, wise old owl. The scope of Medawar's advice is indicated by the titles of his 12 brief chapters: "How can I tell if I am cut out to be a scientific research worker?"; "What shall I do research on?"; "How can I equip myself to be a scientist or a better one?"; "Sexism and racism in science"; "Aspects of scientific life and manners"; "Of younger and older scientists"; "Presentations"; "Experiment and discovery"; "Prizes and rewards"; "The scientific process"; and "Scientific meliorism versus scientific messianism".

There is certainly a great need for a book from which young persons interested in a scientific career but lacking direct access to senior scientists can get some authentic ideas of what it's really like to be in the business. In fact, why hasn't it been done before? And Medawar, who as an adviser to the young sees himself as the Polonius, the Lord Chesterfield or the William Cobbett of science, goes some considerable way towards filling that need. Above all, Medawar emerges from these pages as a role model worth emulating for any aspiring youth: urbane, sensible, open-

Advice to a Young Scientist. By P.B. Medawar. Pp.109. (Harper and Row: New York and London, 1980.) \$8.95, £4.95.



Sir Peter Medawar when a graduate student at Oxford, 1936.

Ramsey and Muspratt, Oxford.

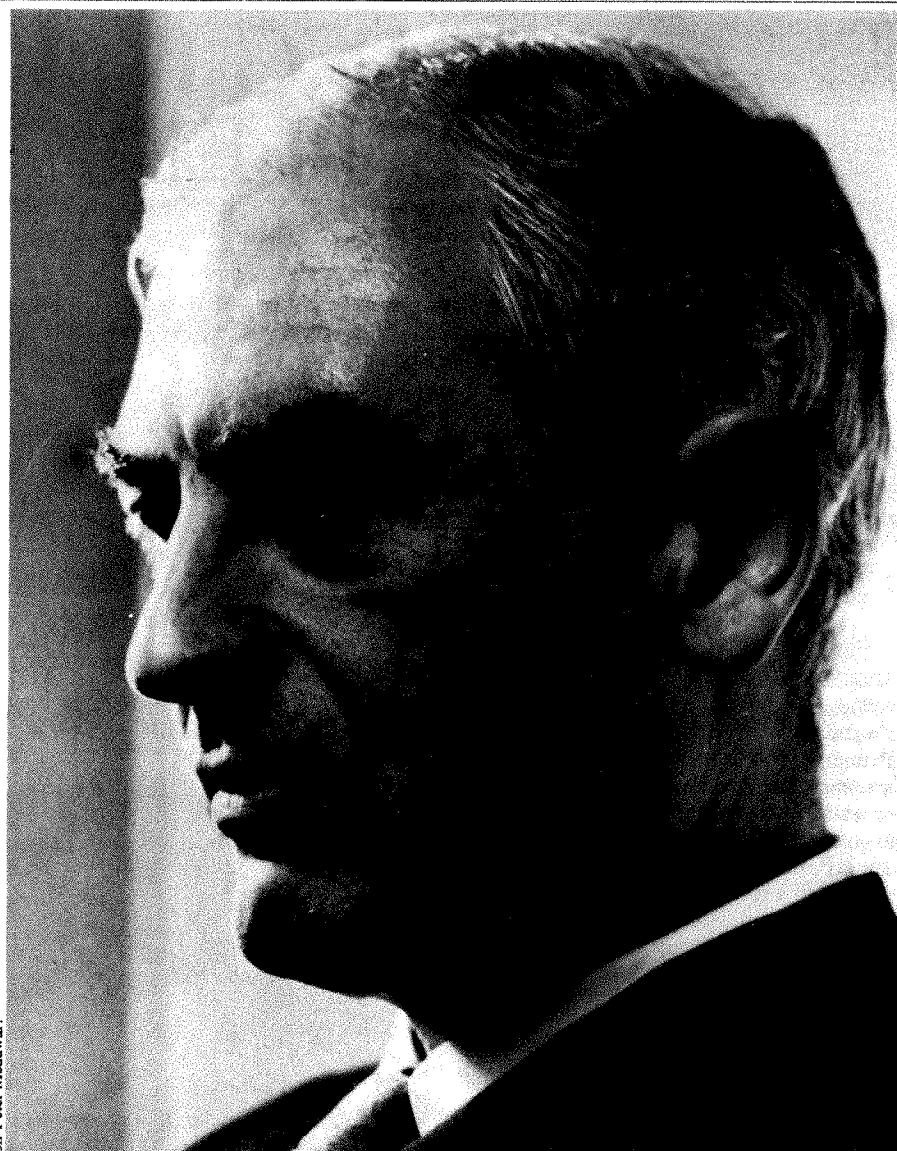
minded and liberal (but, of course, intolerant of nonsense), universally esteemed, and, most importantly, outstandingly successful, having reached the absolute pinnacle of his profession with perfect grace, never clawing his way to the top. Moreover, Medawar provides many *aperçus* of the scientific life, such as that "no working scientist ever thinks of himself as old, and so long as health, rules of retirement, and fortune allow him to continue with research, he enjoys the young scientist's privilege of feeling himself born anew every morning"; that "luck plays a real part in scientific research"; and that "the husband of a [woman] scientist must not expect to find *gigot de poulette cuit à la vapeur de Marjolaine* ready on the table when he gets

home from work probably less taxing than his wife's".

But I do not think the book completely achieves its aims. First, the down-to-earth questions posed in some of the chapter titles are not all given very helpful answers. Thus if the young reader really wants to know if he is cut out to be a scientific research worker, he is provided with little more on which to base his decision than the information that science (just like, say, professional skiing) is a very demanding and sometimes exhausting, albeit exciting and rather passionate, occupation, for which old-fashioned virtues such as diligence, a sense of purpose, and the power to concentrate and persevere are required. Moreover, he should be able to pass an intelligence test provided by Medawar, which consists of discovering what is wrong with the theory that El Greco painted unnaturally tall and thin human figures because of a defect in the painter's vision that made him see people that way. And if the young reader really wants to know what he should do research on, he is given little more guidance than the aphorism that he "who wants to make important discoveries must study important problems". By way of an example of an unimportant piece of research *not* worth doing, Medawar mentions the case of a young zoology graduate student who decided to find out why 36% of sea urchin eggs have a tiny black spot on them. It so happens that I myself am very interested in the role of topographic differentiation of the egg in embryonic development and became very excited on learning that there are black spots on a substantial minority of sea urchin eggs. In fact, I had already worked out the beginnings of a black spot theory of development before I came to the dénouement when Medawar reveals that there are no such black spots and that it was all made up by Lord Zuckerman. And if the young reader wants to know how to equip

himself to be a scientist, he is informed only that he must read the literature (but not too much), that he better get some results (even if they are not original), that he should not waste time building equipment (if it can be store-bought), and, above all, that he ought to practise what Medawar in an earlier book called "The Art of the Soluble" (which is "making a problem soluble by finding out ways of getting at it — soft underbellies and the like").

A second shortcoming of the book likely to trouble intellectually alert young readers is a noticeable lack of logical consistency in some of the propositions put forward by Medawar. For instance, he cautions against "citing Marie Curie as evidence that women can do well in science; any such tendency to generalize from isolated instances will convince no one that they have a natural aptitude for science — it is not Madame Curie but tens of thousands of women gainfully and often happily engaged in scientific pursuits who should be called in evidence". Yet in the same chapter, Medawar merely cites the names of ten brilliant contemporary Hungarians (most of Jewish descent) to demolish the inference drawn from IQ tests administered at Ellis Island before the First World War "that 83 percent of the Jews and 80 percent of the Hungarians seeking entry were feeble-minded". Rather than generalizing from isolated instances, should Medawar not have called into evidence the tens of thousands of Hungarian and Jewish immigrants who did just about as well in the New World as their Anglo-Saxon fellow-citizens? Further, in one place Medawar indignantly rejects the notion that there are any national differences in the character of science — he mentions Japan in particular as one nation whose science has been falsely held to be somehow different from that of the West. In fact, he thinks that such "regional differences are intrinsically unlikely for methodological reasons" and asserts "that no experienced scientist seriously believes that they exist", thus sweeping away with a few strokes of the pen all of Joseph Needham's studies of Chinese science that show that culture *has* a profound influence on the way science is done. Yet a few pages later he makes fun of Fernand Braudel's dictum that history devours the present by calling it (with undoubted chauvinist irony) one of "those profound French epigrams, you know". In another place Medawar declares that "most of the very greatest scientists lived long before Alfred Nobel . . . founded the prize". But some pages later he declares that science has no limits and "will dry up only if scientists lose or fail to exercise the power or incentive to imagine what the truth might be". So, since the vast majority of scientists that ever lived have lived in this century and since their labours have presumably carried us far away from nineteenth century science into the limitless sea of scientific knowledge, the keen young reader will



Sir Peter Medawar

MRC Publications Group

wonder how it is possible that most of the "greatest" scientists happened to have been among that tiny band of old-timers. Is Medawar not giving himself over to what he castigates at the very end of his book as "Arcadian thinking [which] looks not forward nor far away but backward to a golden age that could yet return"?

In this connection it is instructive to examine Medawar's statement that "one can envisage an end of science no more readily than one can envisage an end of imaginative literature or the fine arts". Interpreted literally this statement would merely assert that it is just as easy to envisage an end of literature and the arts as it is to envisage an end to science. But in the context in which it appears in this book the intended meaning of the statement is undoubtedly the claim that it is very difficult to envisage an end to either of these activities. But this claim would be counterfactual since it is, in fact, easy to envisage an end of the sciences and the arts, as was done, for instance, by Hermann Hesse in his *Glass Bead Game*. And it is precisely from envisaging an end of imaginative literature and the fine arts in

our time that the view that most of the very greatest artists lived before our time gains its logical justification.

But these niggling criticisms are not meant to imply that *Advice to a Young Scientist* cannot be read with great profit by young and old alike. On the contrary, the impression that I want to leave is that this book is bound to enlarge any reader's intellectual horizon and give him a good grasp of what it is like to devote one's life to what Medawar calls "exploratory activities of which the purpose is to come to a better understanding of the natural world". For such a life a critical mind is required, which Medawar challenges with the following "chestnut of immemorial origin":

Psychiatrist: Why do you flail your arms around like that?

Patient: To keep the wild elephants at bay.

Psychiatrist: But there aren't any wild elephants here.

Patient: That's right. Effective, isn't it?

G.S. Stent is Professor of Molecular Biology at the University of California, Berkeley.

Re-opening the case for natural theology

Stanley L. Jaki

Creation and the World of Science: The Bampton Lectures, 1978. By A. R. Peacocke. (Clarendon/Oxford University Press: Oxford, 1979.) £10.

A THAW has been developing for the past decade or so in the icy truce between science and Christian religion. Such is the first thesis of the Reverend Dr A. R. Peacocke, Dean of Clare College, Cambridge, in this book, the greatly expanded form of the Bampton Lectures for 1978. Of course, there were some tell-tale events well antedating the 1970s. It was a quarter of a century ago that Julian Huxley chose to drop from the second edition of his *Religion without Revelation* a crusading preface, his clarion call to all men to rid themselves, once and for all, of the illusion of being dependent on a personal God. Such a call is almost invariably orchestrated in that major key which is the presumed irreconcilability of science and religion and which is aimed primarily at the Christian creed, the major form of belief in God in the Western world, the cradle and powerhouse of science.

As to the past decade, it has widely witnessed the survival value of religion without revelation, after the late Jacob Bronowski refused to heed a suggestion of BBC Television to let them produce his *Ascent of Man* without its anti-Christian innuendos. Religion without revelation could, if the second Bronowski-lecturer (Philip Morrison) was right, be developed by termites, who, given enough time, perhaps billions of years, would come up, so he claimed, with a telescope worthy of Palomar. Like-minded pundits are no longer the exclusive voice of the world of science. Indeed, not since the days of a 'holy wedlock' between science and Christian religion (the days of Boyle and Newton) has it been as fashionable for scientists to refer to creation as is the case nowadays. Peacocke avoids following them into taking the 2.7°K cosmic background radiation for a measuring device of time elapsed since the moment of creation. At any rate, cosmology (the basic science, if all science is cosmology — a proposition endorsed by Popper and with no touch of falsifiability) is not among the major sources, surveyed by Peacocke, of the thaw in question. He rather emphasizes the rising awareness among scientists of the revisability and socio-psychological conditioning of their assumptions and theories. Another major source is that

ecological concern which forced the scientific community to face up to questions of ethics, often germane to a theological perspective.

Therefore the times are propitious, Peacocke argues, for re-opening the case for natural theology. Indeed, its thorough recasting is in order, if it is true, and Peacocke, a biochemist by first training, has no doubt that science has clarified all basic questions about all forms of life, including man, by showing that all are but 'living matter', so many complex agglomerates of atoms and molecules. Thus, if for the Christian theologian the world is to remain a 'launching pad' towards its Creator, the world must be perceived through lenses 'ground and polished by science' (page 48).

Peacocke does not have in mind a stance of 'genueflecting to science', a stance anyhow incompatible with the stipulations of the Reverend John Bampton, Canon of Salisbury. The Bampton-lecturer, a scholar in Anglican orders, must preach 'eight Divinity Lecture Sermons' on any of six specified topics, all of which restrict genuflection before the Triune God alone. Since the last of those topics, 'the Articles of the Christian Faith, as Comprehended in the Apostles' and Nicene Creed', rests on the dogma of the createdness of all things visible and invisible, a thorough confrontation of science and Christian faith is unavoidable, if the latter is to remain a 'reasoned service', a stipulation of the Apostle Paul. Such a confrontation is meaningful only if the merits of the respective claims of science and of (Christian) religion are subjected to a thorough scrutiny, which touches not only on the very foundations of Christian theology but also on those of science. Therefore an undertaking of this sort must be of considerable interest to those natural-scientist readers of *Nature* who cherish vistas of science extending far beyond their often extremely circumscribed specializations.

The theme of thaw and its background, which is the topic of Chapter 1, is followed by 'Cosmos, Man and Creation' in which the shift from the world picture of classical physics to that of modern physics, the interplay of chance and necessity, and the Judeo-Christian doctrine of creation are dealt with. Chance is again considered in Chapter 3, and with special attention to the ideas of Monod, Prigogine and Eigen on emergent systems. Peacocke's discussion of the mind-body relationship, the substance of Chapter 4, may have come more logically after his discussion of evolution ('The Selfish Gene and What Men Live By'). All these chapters certainly should keep alive the interest of scientists.

Chapter 5, in itself a small book, is strongly theological, which is not to say that it is uninteresting. Peacocke's treatment of man's ethical nature and of Jesus' life and resurrection will delight those who savour process theology taken in

a broader sense. Peacocke explicitly dissociates himself from neo-Thomists and Barthians. His predilection is rather with process theologians who showed marked interest in science and who published mostly during the past decade. While attention to the latest is certainly a great merit, it may also trap one in what Duhem memorably described as the 'gossip of the moment', hardly ever the harbinger of depth. Is it really a sign of profundity to give, as Peacocke does, so much and sympathetic attention to a recent interpretation of 'Yahweh' as meaning 'He who makes things happen' and ignore at the same time its long-standing interpretation as Existence Itself, an interpretation that played a crucial role in the history of Western thought? At any rate, if the provenance of 'Yahweh' must be sought in the political experience of the people, the question arises as to why other Semitic tribes, equally battered, failed to formulate it? Peacocke does not face up to this question, nor does he take a recourse to revelation, which incidentally, is mentioned only once throughout the book. A curious feature, because the last two chapters, on ecology and hope, respectively, are pivoted on the uniqueness of Jesus which Peacocke portrays over half a dozen, not too impressive pages, and because it is his stated view that in his lectures 'there are involved . . . a sequence of theological themes . . . that echo credal phrases from the Book of Common Prayer' (page ix).

Those phrases, from creation through redemption to final consummation, take on, as articulated by Peacocke, a distinctly phenomenological ring. This is not to suggest even the slightest doubt about his deep commitment to the Creed. But no doubts could be raised on that score about Henry Longueville Mansel, author of *The Limits of Religious Thought*, the Bampton Lectures for 1858. Yet, an Oxford don, apparently of some stature, was heard to remark: 'I had not expected to live to hear atheism preached from the pulpit of the University' (see V. F. Storr, *The Development of English Theology in the Nineteenth Century 1800-1860*; Longmans, Green: London, 1913; page 422). Not that Mansel would have wanted to give the slightest comfort to atheists, or to anticipate a religion without revelation. But as that perceptive don realized — and long before the inner logic of Mansel's highly applauded lectures dawned on all — the premises on which he built could not accommodate a reasoned account of Christian revelation. Classic illustrations of the inexorable force of that logic can be gathered not only from theology and philosophy, but also from the sciences. The road is straight from Descartes to De la Mettrie, from Luther to Schleiermacher, from the early Copenhagen school to the multiworld-theory and the Tao of physics.

An ominous-looking logic may raise its head out of Peacocke's position on the

mind-brain relationship, an inevitable consequence of his unqualified endorsement of the philosophy of emergent systems based on the work of Prigogine and others. To throw a strong light on this possibility, it may not be amiss to recall a remark which Herbert Feigl, the chief modern articulator of the identity theory of the mind-body relationship, made to the author of a book in which the case for dualism is unabashedly argued throughout: "Of course, I could not disagree more with you. Unfortunately I cannot do even that when reading some professedly Christian theologians on mind and body. I am unable to comprehend their contention that their position is different from mine". Needless to say, Feigl's position is compatible only with a religion without revelation as meant by Huxley. As to dualism, which Peacocke considers only in the perspectives of demonology and witchcraft, it is, in his eyes, an invitation to physicalist reductionism. This is certainly true of that parody of dualism produced by Descartes, a point not specified by Peacocke, who fails to mention even passing other formulations of dualism. He opts for the identity theory, which he introduces with appreciative words on materialism and monism. His sole effort to retain the mind (soul) is a brief reference to the conceptual irreducibility of mental experiences to empirical parameters.

This is hardly enough, as would be noted by those taking a long look both in Pusey Street and South Parks Road, respective symbols, in Peacocke's phrasing, of theological and scientific strongholds. They would point out that to discourse on an issue so crucial both to revealed religion and to religion without revelation as is the true nature of the mind-brain relationship, it is not enough "to note from the stands certain aspects of the state of the play". Peacocke states in the same breath: "I would not presume to enter the lists of that fearsome tiltyard from which so many, more competent and distinguished philosophically than I am, have retreated to lick their logical wounds" (page 128). Wounds cannot be escaped as long as one truly joins the battle for truth. At most they can be kept disinfected by making one's position clear and thoroughly considered.

The absence of these two qualities undercuts time and again Peacocke's efforts. A subject like creation and the world of science brings one into encounter at every turn with that philosophy which cannot be done justice with the evasive remark that the case for a qualified realism, adopted in these lectures, "cannot be presented here" (page 22). This transparent tactic is in sight again after a long section on time as perceived in science (evolution) and in theology (eschatology): "The philosophical discussion of time is beyond our present scope" (page 334). Even natural-scientist readers, not overly sensitive to philosophical rigour, may feel uneasy on finding Peacocke declare in the

next-to-last chapter that he had until then used the word nature "without any attempt to define it". After all, he himself tells his readers that several decades ago two scholars listed no less than sixty-six meanings of "nature", a number that could since then only increase.

A perhaps trivial, but telling sign that Nature is not static. Of this, Peacocke's book is a spirited and well-informed reminder. But in order to mean anything, Nature, and certainly man's nature, must retain some identity across the welter of change, especially if change is a true growth. Had Peacocke considered this basic philosophical issue, the touchstone of the truth of realism, with the seriousness it demands, his never-dull effort to portray

the dynamism of world and man as "being-in-God" would have secured more persuasiveness for *pan-en-theism*, the label by which he wants his message to be known. The proofs of his book may have been read with unusual speed, though, undoubtedly, not with an eye on Canon Bampton's final stipulation: "the Preacher shall not be paid, nor be entitled to the revenue, before his sermons are printed." □

Stanley L. Jaki is Distinguished University Professor at Seton Hall University, South Orange, New Jersey, and served as Gifford Lecturer at the University of Edinburgh (1975 and 1976) and Fremantle Lecturer at Balliol College, Oxford (1977).

Lives of the stars

J.H. Manley

The Uranium People. By L.M. Libby. Pp. 384. (Crane, Russak/Scribner's: New York, 1979.) \$15.95. To be published in the UK in May 1980 by Adam Hilger, at £8.50.

It is easier to convey what this book is not than what it is. It is not a novel though it would be better and less offensive if it were. It is not in the category of memoirs, for although there are personal recollections, there is also a liberal use of the personal accounts of others. The reader will find the distinction between these two sources very un-sharp and will slowly learn that the geographical chapter headings have more to do with the subject treated than the location of the author. It is not a historical work in spite of the impression conveyed by the inclusion of references. Even these are inconsistent

with a historical work. Some quotes are unreferenced; practically none to official papers and compilations are included. In addition one is aware throughout of the intrusion of the author's opinions and tastes in people and events rather than the balanced pro and con of the careful historian or the insightful analysis of the biographer. The fact that the book grew out of a series of lectures as a university visitor suggests that the material and style were meant to be entertaining to students. I believe they were. There are many anecdotes, some amusing, some instructive, some flat. The author's statement of her purpose is to "tell not only what was scientifically interesting about the uranium-plutonium project but what was human and interesting about the people involved". Let us therefore consider her treatment of people and events.



Enrico Fermi (left) and John Marshall, 1946.

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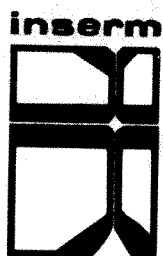
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The title suggests an emphasis on people and the text starts that way. I confess to disappointment in Libby's efforts to describe people. Practically all the individuals for whom she implies a personal acquaintanceship are also known to me, some less well, some better, but I did not recognize them very well as portrayed. I finally concluded that at least part of the reason is her often tasteless and tactless choice of anecdotes used to suggest interest in so-and-so. There is a strained and misguided effort to make people seem more human by calling attention to uncommon mannerisms or recounting stories which are easily called crudities of various degree. Mention of an instance in which Laura Fermi listed the inner organs of a turkey says nothing about her graciousness. The worst crudity, an episode used to explain a facet of graduate student collective mores, involves the death of a colleague. To a point, foibles help to visualize a person and may make him interesting, but other less curious personality descriptions are needed for balance. These are missing, and even the non-anecdotal information is confined largely to physical characteristics. Rarely are adjectives relating to character used. In one case she explains that a person has been called a "delta-function of sympathy" but even an included definition of that term does not bring much of the person to the reader. In the sense that common gossip more frequently delights in communicating less desirable human traits as well as being idle, many of Libby's descriptions are gossipy. The less favour in which she holds a person the more gossipy the material. Leo Szilard as an individual is not crystallized by a good explanation of the Szilard-Chalmers effect. Fortunately the inclusion of a quote about him from Jacques Monod contributes excellently. Similarly, General Groves becomes more real through a quote from Emilio Segrè.

One could wish that at least a few of the people might have received the kind of thorough, thoughtful and sensitive treatment Nuell Pharr Davis achieved in *Lawrence and Oppenheimer* (Simon and Schuster: New York, 1968). The difference is all the more remarkable when one realizes that Libby lived and worked with many of her people, but Davis became acquainted only by interview, albeit with an impressive list of individuals. *The Uranium People* is a misleading title. It is more about events and stories of people, with little attempt to examine the humans at any length or the subtle reasons why they so frequently found each other interesting.

Events fare better in this book than people. Descriptions of places though not extensive are well written and convey correct images; so with technical explanations, some of which may not be essential to the lay reader. With few



From left, Ernest Lawrence, Enrico Fermi, J.I. Rabi at Los Alamos lodge.

exceptions I found no serious errors of historical or scientific fact. Use of many interesting memories of the author and of others makes a readable story. As with people, the interpretation of events is well tinted by the author's personal preferences and opinions.

Scientific-military-industrial roles in the project are discussed in several places, most frequently in terms of good scientists and bad military and industrial people, though not consistently so. One episode described contains speculation on a possible not-quite-right cosiness between General Groves and the engineering firm of Stone and Webster. This gets confusedly related to Arthur Compton and God, to a belief of a number of Metallurgical Laboratory scientists that industry need play only a minor role and to a jibe at Groves for depriving Compton of responsibility for weapon research and development in favour of Oppenheimer. It was surely naive of the scientists (including the author) to think that the production reactors and extraction facilities could be designed and built by them with a few hundred engineers and draftsmen. Libby implicitly acknowledges this in a later description of the DuPont achievement of completing this technical task including a city to house 60,000 construction workers all in 18 months. However, she has no appreciative word for Groves for his wise and effective choice of this contractor. The account of Groves' shift of weapon responsibility is quite wrong. After May 1942 Oppenheimer and I were sharing responsibility for the geographically scattered weapons work under Compton.

In a few months it became very clear that the work would be hopelessly behind unless it were centralized in a new laboratory with proper facilities. Compton concurred completely and fully supported our view to Groves. It was obvious that he could not carry the responsibility for two widely separated laboratories; a search for a Director was initiated and Oppenheimer chosen with complete approval of Compton. I felt he was glad not to have the weapon project.

The last chapters of the book cover such events as the establishment of the Atomic Energy Commission, the Russian A-bomb, and the H-bomb controversy and aftermath; and there is a chapter on atomic power. The style of writing continues, perhaps intensifies. Groves remains a bad guy until she can report his departure from the atomic scene with words symptomatic of her approach: "General Groves had put down Arthur Compton. Now his own boys, Robert Oppenheimer, Bush, and Conant had put him down. And soon, Oppenheimer was to be put down, in turn, by the Grey Board". Gentle, understanding, sympathetic! Libby finds it "hard to understand how the General Advisory Committee, chaired by Oppenheimer, could have persuaded itself to have voted negatively on the question of whether the United States should make an urgent programme to develop the thermonuclear bomb". At first one thinks that perhaps she did not pay enough attention at the time and then later failed to read Herbert York's careful and extensive examination of this and associated issues in *The*

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Advisors (Freeman: San Francisco, 1976). Further on, however, she has included a selective quote from him but not his conclusion that the advice of the Committee was "sound and right". She also writes: "One may ask how the GAC members, strongly opinionated scientists, could have so changed their vote on the hydrogen bomb in a couple of weeks". Reference is then made to a meeting on 29 October and then to a "second meeting". Actually, there was no change of vote, and the written views, unanimously against an all-out effort, are dated 30 October. If entertainment is the objective of Libby's book, perhaps these details are unimportant, but I view such distortions as poisonous. The student and the serious reader must take something like York's book as an antidote; then Libby's continued loyalty to "one of my heroes", Edward Teller, can be set in contrast to York's development from his early career as the first director (1952-57) of Teller's

Lawrence Livermore Laboratory to his subsequent activity in arms control and disarmament.

The Uranium People is interesting in the same sense that movie magazine stories about the stars are interesting. However, events and people, now for the most part passed into history but leaving a legacy which still affects our world, have a different kind of interest and deserve a different kind of treatment. □

J.H. Manley became active in nuclear physics at Columbia as a faculty member with I.I. Rabi, John Dunning and others in 1934, and joined the Metallurgical Laboratory at Chicago under A.H. Compton in January 1942 while on leave from Illinois. He assisted J.R. Oppenheimer in planning and establishing the Los Alamos Scientific Laboratory, to which he moved for what turned out to be a total of 23 years before becoming a consultant — his present status. He was Executive Secretary of the General Advisory Committee (USAE) during the first four years of Oppenheimer's chairmanship.

Descent is what matters

David L. Hull

The Darwinian Revolution: Science Red in Tooth and Claw. By Michael Ruse. Pp.320. (University of Chicago Press: Chicago and London, UK, 1979.) £12.

MOVIE reviewers like so few movies because they see too many of them. For the same reason, academics write very few favourable reviews of books, especially if the book is in their own area and is directed at a general audience. It is difficult to believe that yet another book on Darwin and the Darwinian revolution could add anything new or contain any surprises. Ruse's book is an exception on all counts. Darwin scholars and the general reader alike can learn from it.

In the first half Ruse describes the state of science before the *Origin*. Historians argue that scientific views must be set out in their own terms, not biased towards the use which later scientists will make of them. That is easier said than done. Ruse cannot discuss every topic of interest to scientists in the first half of the nineteenth century. Understandably he limits himself to those areas of science which eventually contributed to Darwin's theory of evolution — Charles Lyell's uniformitarian geology, Richard Owen's archetypal theory, Karl Ernst von Baer's embryology, and so on. In certain cases, Darwin incorporated these views into his own. Just as often his ideas were shaped by

reacting against the views of his predecessors. But, in any case, once Ruse has narrowed his focus, he discusses the science in its own right, not biased towards the use which Darwin eventually made of it. Although Ruse teases us a bit along the way, he does not get around to Darwin and his theory until the middle of the book. All the attention which Ruse pays to the scientific work which preceded Darwin, instead of detracting from the magnitude of Darwin's own contributions, only enhances it.

Another strength of Ruse's exposition is his concern with the impact that social factors had on both the formulation and the reception of Darwin's theory. However, the 'society' of greatest interest to Ruse is not Victorian society at large but the society of scientists. Darwin was caught up in the groundswell as science in Great Britain became self-consciously a profession. Young scientists today can only envy the ease with which budding young scientists in Darwin's day were able to step into the innermost circles of science. To begin with, relatively prosperous Victorians were a tightly-knit group. For example, the first three directors of Kew Gardens were William Hooker, his son J.D. Hooker, and William Thiselton-Dyer, the younger Hooker's son-in-law. J.S. Henslow was the first scientist to have significant influence on Darwin, and J.D. Hooker was his first convert to evolutionism. Henslow married the sister of Leonard Jenyns, the man who had been offered the position of *de facto* naturalist on the *Beagle* before Darwin. Their daughter in turn married J.D. Hooker. Upon her death, Hooker married the widow of William Jardine, a famous ornithologist. Darwin married a Wedgwood,

and so it goes. Of equal importance, so few young men were inclined or financially able to contemplate a life of science that the ones who did were welcomed enthusiastically.

Darwin began his career as a Lyellian geologist. In retrospect we tend to think that Lyellian uniformitarian geology was more successful than it actually was because the first histories of geology were written by uniformitarians. To the victors goes the history. But in fact Darwin was one of the few young geologists at the time who accepted Lyell's views. Darwin's early work on the formation of coral reefs (which turned out to be basically correct) and the parallel roads of Glen Roy (an embarrassing blunder) was in the Lyellian mould. Gradually, however, Darwin became his own scientist, departing in several important respects from Lyell. By 1859 not only was Darwin a professional scientist with a well established reputation, but also he had "built around himself a new scientific community from which his evolutionism could be launched" (page 185).

One of the great puzzles in science is why certain scientists succeed in converting their fellow scientists when all their precursors failed. For example, Lamarck's theory was greeted with derision, Robert Chamber's *Vestiges of the Natural History of Creation* was condemned by everyone who was anyone, including T.H. Huxley, and Herbert Spencer's musings about the transformation of homogeneity into heterogeneity produced little more than embarrassed silence. Ruse gives many reasons for Darwin's success — such general considerations as the changes wrought by the industrial revolution and the rise of naturalistic metaphysics to such specific considerations as the way in which Lyell posed the species problem in his *Principles of Geology* — but of prime importance was the professional manner in which Darwin conducted himself and presented his case. Lamarck had destroyed his credibility by tossing out theory after theory in areas in which he knew nothing; Chambers was the Velikovsky of his day; and Spencer was a "philosopher". But Darwin was a scientist, writing as a scientist, for scientists. Nineteenth-century views on the nature of science had as much to do with the reception of Darwin's theory as religion and the fossil record.

The most entertaining part of Ruse's exposition is the attention he pays to the interpersonal relations between the scientists of the period. Although Owen initially helped the young Huxley in his career, he soon came to be Huxley's chief enemy. Because the Darwinians won, everyone knows what an unpleasant man Owen was, but Huxley too had a mean streak. After Owen tried to block the publication of one of Huxley's papers, Huxley used his review of the *Vestiges* to attack him. As Ruse sees it, the animosity between Huxley and Owen was as much

responsible for Owen coming out against Darwin as the content of Darwin's theory. "At the scientific level Owen was determined to oppose Huxley, and if Huxley was to support Darwinism then so be it" (page 144). Ruse also mentions, possibly to neutralize anti-Owen propaganda, that "at least until 1853 — when Huxley started attacking Owen and when Darwin and Huxley became close — there was warm feeling between Darwin and Owen" (page 144). The source of this claim is the following remark which Darwin made in a letter to Huxley in 1854 about Huxley's review of the *Vestiges*:

By Heavens, how the blood must have gushed into the capillaries when a certain great man (whom with all his faults I cannot help liking) read it!

This is warmth of sorts.

One of the chief messages of evolutionary theory as a biological theory is that descent is what matters, not abstract similarity. Ruse applies this same principle to the history of science. No scientist has suffered more from the effects of the antiquarian disease known as "precursoritis" than has Darwin. Endless energy has been expended to uncover precursor after precursor — authors who happened to mention in passing one or more views similar to those later enunciated by Darwin. More recent historians have complained that often the putative similarity is spurious. But the important point is that ineffectual precursors do not count. As Ruse argues, "it would be a mistake to think that the most significant intellectual continuity in the Darwinian Revolution existed among those one would label 'evolutionist'" (page 199). The real precursors to Darwin were not Lamarck, Erasmus Darwin, William Wells, Patrick Matthew and Edward Blyth. Darwin was totally unaware of the work of several of these men, and the others had little effect on him or anyone else. Darwin's real precursors were Lyell, Henslow, Owen, Herschel, Whewell, von Baer and Sedgwick. These were the men who contributed to the Darwinian revolution. What counts in the history of science is continuity of effect, not similarity of ideas. Likewise, as Ruse remarks, a "Darwinian" is "someone who identified with Darwin but not necessarily someone who accepted all of Darwin's ideas" (page 203).

During the past decade, Darwin scholars have increased our understanding of Darwin and the Darwinian revolution immensely. Ruse gives his readers the benefit of that scholarship without making them suffer under the burden of the tedious pedantry which usually accompanies it. □

David L. Hull is Professor of Philosophy at the University of Wisconsin-Milwaukee, Milwaukee, Wisconsin.

Full of hope and promise

R.V. Short

The Politics of Contraception. By Carl Djerassi. Pp.274. (W.W. Norton: London, 1980.) £6.50.

CARL DJERASSI, one of the world's outstanding organic chemists, has written a most important book about contraception that makes compelling reading. It deserves the widest possible audience, not only throughout the scientific community, but far beyond, in the realms of policy-makers, politicians, journalists, administrators, funding agencies, and indeed amongst all who are concerned about world futures. There are few indeed who have mastered the art of popularizing important issues without seeming to trivialize them; this book stands out as a rare example of scientific reporting at its very best. Djerassi's writing reflects the man himself — forthright, scholarly, direct, uncompromising, and with an outstanding ability to single out important issues for critical comment. He shares with Samuel Pepys "a fine conceit" that adds to, rather than detracts from the narrative, because one respects the integrity and intellect of the man behind the pen.



Carl Djerassi.

Djerassi tells us that he has aimed to produce "not a scholarly treatise, but the distillate of the conclusions and opinions of a person who for over 20 years has lived a bigamous professional life in serving simultaneously as a professor carrying out basic research and as an industrialist who has had to concern himself with finding worldwide applications for laboratory discoveries". Not only has he been able to combine the viewpoints of a professor of organic chemistry with those of a director of a pharmaceutical company, but he has added profound biological and social insights; it is to this amalgam of talents that we must attribute his success as an

international entrepreneur, and the wide appeal of this particular book.

Djerassi will long be remembered for an article he wrote in *Science* in 1970 (*Science* 169, 941-951) entitled "Birth control after 1984", and his book is an extension and development of the ideas put forward in that article. The need to redouble our efforts in contraceptive research and development is self-evident at a time when the world's population is still continuing to increase by about 150,000 people a day; this single fact still continues to pose the greatest threat to the peaceful co-existence of mankind in the future.

Our principal goal must be to develop new approaches to contraception capable of deployment on a massive scale to meet the needs of the developing world; of relatively minor importance is the improvement of methods that we already have in the West, so as to maximize their efficacy and minimize their attendant health hazards. But unfortunately it is our obsession with these latter objectives in developed countries that has seriously impeded research into new contraceptives. Much of this research must inevitably be carried out in the West, because it is only in the developed countries that we have all the necessary facilities, funds, and trained minds, together with the industrial know-how to exploit the discoveries. Herein lies the dilemma. In the first place, the declining rate of population growth in Western nations has allayed public and political concern for the problem, so that governmental support for research has now switched to more immediate problems such as alternative energy sources. This change in emphasis has coincided with the development of a plethora of governmental regulations about drug safety, and the establishment of a bureaucracy to enforce them. The statutory requirements for long-term animal toxicity testing before a drug can be marketed means that it may take from 10 to 17 years between the discovery of a compound, and its approval for clinical use by the regulatory agencies. Thus it is hardly surprising that new product development in the contraceptive field has become one of the least attractive areas of research for the pharmaceutical industry; but without the active involvement of industry, our hopes of any significant new contraceptive developments are faint indeed.

In our national drug regulatory agencies we have created organizations who have absolute power, but no sense of responsibility to see that a compound that is desperately needed is ultimately marketed; witness the status of the injectable contraceptive Provera. Surely we need to establish some mechanism whereby the pharmaceutical industry and the regulatory agencies could have a common cause once it has been agreed that there is a need to develop drugs to meet a particular demand. Under the present system, the regulatory agency acts as State

prosecutor, and private industry is the defendant. The case only comes to trial after years of time and effort and, as Djerassi points out, the pharmaceutical company does not have a friend in court. Surely there would be much to be gained by exchanging the trauma of a final examination for some type of continuous assessment?

Although agreeing with the need for governmental regulations about drug development and safety testing, Djerassi highlights the bureaucratic stupidity of some of the consequences. For example, the US Food and Drug Administration's requirements for condoms have meant that America produces the thickest condoms of any country in the world; if standards were relaxed to permit a 1% increase in the incidence of pin-holes, condom thickness could be reduced by half, and the increased risk of pregnancy resulting from this would only be one conception per 2.5-5 million acts of coitus. The instructions that now must accompany each packet of oral contraceptives are almost unintelligible even to the expert, and Djerassi contrasts this to the simple and effective wording that is used in China. Indeed, the chapter devoted to birth control in China is one of the most fascinating and challenging in the whole book.

Djerassi inevitably paints a rather gloomy picture about our ability to develop those new approaches to contraception which we so desperately need. But he may be unduly pessimistic, as he has based his strategies for the future on a logical planned approach of contraceptive research and development, and has ignored the major role that accident and chance can take in such affairs. For example, we can derive some comfort from the fact that in

reviewing probable future developments in the field, Djerassi does not even mention what to many seems to be the most exciting hope for the future, the use of analogues of gonatrophin releasing hormone. It has recently been shown that daily administration of microgram amounts of one of these peptides can effectively inhibit ovulation for long periods of time in monkeys and in women. Here we have an example of a serendipitous discovery; these readily synthesized, non-toxic, non-steroidal compounds were developed by the pharmaceutical industry for the treatment of some forms of infertility, but paradoxically they can also be used as contraceptives. As they have already passed many of the drug regulatory agencies' hurdles, and are on the market in some countries, they could be available for widespread clinical use within a few years from now.

So the future is full of hope and promise, provided that the West can increase its funding for contraceptive research, and remove the existing bureaucratic impediments to commercial development. But there are many obstacles to be overcome if we are to develop a rational approach to contraceptive research and development, and the world must be indebted to Carl Djerassi for pointing out some of the illogical constraints we have imposed upon ourselves. There can be no doubt as to the truth of his general conclusion that it is the politics of contraception, rather than the science, that now plays the dominant role in shaping the future. □

R. V. Short is Director of the Medical Research Council Unit of Reproductive Biology, Edinburgh, UK.

The micro future?

Stuart Sutherland

The Mighty Micro. By C. Evans. Pp. 255. (Gollancz: London, 1979.) £5.50.

OVER the past 20 years, many voices have noisily if somewhat prematurely hailed the advent of the computer revolution. It has had no more enthusiastic advocate than the late Christopher Evans, whose premature death occurred after *The Mighty Micro* had been published. It has for long been customary to place the revolution about two decades after the date at which the prediction is made, and Dr Evans adheres to this convention. By AD 2000, we will have, according to him, universal electronic mail, greatly improved Prestel sets on our wrists, and permanently worn silicon chips to monitor our health and automatically inject corrective drugs as needed. Money will have been abolished

since all financial transactions will be computerized, and computers will administer psychotherapy and justice as well as taking over the function of the teacher: bookshops and libraries will no longer exist, since it will be possible to call up anything ever published on your drawing-room VDU.

The Mighty Micro is written for the general public, not for the specialist. It is lively and entertaining, but makes no attempt to describe how a silicon chip works nor does it provide any insights into the intricacies of computer software. It contains some good anecdotes about the forerunners and pioneers of computing — Babbage, Hollerith, Zuse and Schreyer (who developed special-purpose computers in Germany in the Second World War), Aitken, Turing and Von Neumann. The pioneers saw a visionary

Society for Applied Bacteriology Symposium Series No.8

Microbiological Classification and Identification

edited by M. Goodfellow and R.G. Board

June/July 1980, xiv + 396 pp. £17.50 (UK only)/\$40.50 0.12.289660.2

This volume, the eighth in the SAB Symposium Series, answers the need for a comprehensive examination of the impact of modern taxonomic methods on the classification and identification of important groups of organisms. It offers, for the first time, a coherent view of a difficult subject of considerable significance. The information is detailed and up-to-date, and covers methods such as numerical, genetical and chemical taxonomy. It highlights the value of nucleic acid homology and base ratio determinations, and considers the contribution of biochemical and morphological techniques. The significance of plasmids on the process of classification and identification is also discussed. The chapters are written by leading specialists from a variety of disciplines; their contributions will stimulate interest far beyond the confines of these particular areas, and will help to re-establish taxonomy as a deserving field of research in its own right.

Probability and Mathematical Statistics: A Series of Monographs and Textbooks

Statistical Inference for Stochastic Processes

I.V. Basawa and B.L.S. Prakasa Rao

June/July 1980, xvi + 438 pp. £26.80 (UK only)/\$62.00 0.12.080250.3

The current decade has witnessed an exponential growth of literature on stochastic processes. However, much of the work seems to be dominated by theoretical models which lack immediate appeal from a statistical point of view, and as a result a gap has developed between theory and applications. The aim of this book is to reduce this ever-widening gap by directing the interest of researchers in the area to the inferential aspects of stochastic processes. The authors have here attempted to put on record the work illustrating the current state of the art.

Monographs of the Physiological Society No.37

Local Mechanisms Controlling Blood Vessels

W.R. Keating and M. Clare Harman

June/July 1980, viii + 142 pp. £11.80 (UK only)/\$27.00 0.12.402850.0

Research undertaken during the last fifteen years has shown that blood and lymphatic vessels, and mammalian arteries in particular, are controlled by much more organised and complex local mechanisms than had previously been realised. These include specialised functions of inner and outer smooth muscle, as well as electrical discharges with unusual features. These have only recently become clear enough to allow any coherent general analysis. This book aims to present such an analysis in a brief and convenient form, and provides extensive references for readers who wish to re-examine controversial details of the evidence.

Society for Applied Bacteriology Technical Series No.15

Microbial Growth and Survival in Extremes of Environment

edited by G.W. Gould and Janet E.L. Corry

June/July 1980, xvi + 244 pp. £16.00 (UK only)/\$37.00 0.12.293680.9

This volume presents the contributions made at the Autumn Demonstration Meeting of the Society for Applied Bacteriology in October, 1978. It brings together current research and modern methods and techniques used by microbiologists and biochemists dealing with diverse research problems, but sharing an interest in microbial growth and survival under extremely unfavourable conditions. The extreme environments discussed vary greatly in type, ranging from hot to cold, acid to alkaline, low to high water activity and raised osmotic pressure, dry, saline and sugar-rich. Other papers are concerned with chemical and biochemical extremes, including high levels of toxic chemicals and hyperbaric oxygen. The somewhat neglected, but ecologically most important, environmental extreme of low nutrient concentrations in oligotrophic waters is also considered.

Biological Microcalorimetry

edited by A.E. Beezer

June/July 1980, x + 484 pp. £30.00 (UK only)/\$69.00 0.12.083550.9

Biological Microcalorimetry is an up-to-date account of microcalorimetry as applied to biological topics and gives a comprehensive review of this growing area of research. For non-calorimetrists it is intended as an introduction and for those already involved in calorimetry it provides a survey of other important biological studies. The topics covered range from 'pure' model biochemical systems to complex, intact biological systems such as blood: also included are discussions of both practical and theoretical problems. This book reflects the way in which research activities have changed and also the extent to which practising biologists have accepted calorimetry. The scope is wide and includes microbiological systems, tissue cells and drug interactions. There is, too, a concentration upon model studies, for example membrane, polymer and heat capacity studies.

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Proceedings of the Sero Symposium No.29

Pituitary Microadenomas

edited by G. Faglia, M.A. Giovanelli and R.M. MacLeod

June/July 1980, xii + 565 pp. £30.00 (UK only)/\$69.00 0.12.248150.X

The importance of intrapituitary adenomas was established in 1932, although considerable progress has been made only during the last few years. Topics covered in the book include morphological and molecular features of hormone secretion in normal and tumoral cells in the pituitary, immuno-histochemical aspects, and pathogenesis of pituitary tumours. The discussions also deal with the study of receptors, the role of neurotransmitters in the control of pituitary functions, and neuroendocrinology and endocrinology of pituitary microadenomas. The diagnostic studies include endocrine function tests and radiology, while microneurosurgery, cryosurgery, alpha particle therapy and neuroactive drugs are treated in the papers on therapeutic methods.

The Bipyridinium Herbicides

L.A. Summers

June/July 1980, x + 450 pp. £30.00 (UK only)/\$69.00 0.12.676450.6

The bipyridinium herbicides, diquat, paraquat and related compounds, have attracted considerable interest over the past twenty years. This is the first book to be devoted solely to this important group of herbicides; it provides a comprehensive and up-to-date account of the published work in this area, and aims to put all aspects of their study into perspective. The book cites approximately 2500 references. The synthesis, chemistry, activity and applications of the herbicides are discussed in full, and toxicology, plant physiology and the mode of herbicidal action are also treated. These discussions involve a wide variety of disciplines. It will prove a valuable reference to all those interested in the bipyridinium herbicides, and particularly to researchers involved in chemistry, biochemistry, biology, agricultural science, soil science, toxicology, medical science, and environmental science. It should also be of interest to students as supplementary reading in these fields, and will be a necessary addition to many institutional libraries.

An Introduction to Spectroscopy for Biochemists

edited by S.B. Brown

May/June 1980, xvi + 404 pp. £16.60 (UK only)/\$38.50 0.12.137080.1

Over the past fifteen years there have been major developments in the application of spectroscopic methods to systems of biological and medical interest. Biochemical literature increasingly embraces spectroscopic techniques such as nuclear magnetic resonance, electronic spin resonance and circular dichroism. This book aims to provide a basis for understanding and evaluating such literature, and to create an awareness of the established and potential value of spectroscopy in solving problems. The principles of each technique discussed are presented in a straightforward and precise, yet largely non-mathematical style. Emphasis has been placed on a wide survey of the application and scope of the various branches of spectroscopy, which include vibrational, molecular emission, ultraviolet and visible, and atomic absorption, while references to further works are given at the end of each chapter.

Carbohydrate Sweeteners in Foods and Nutrition

edited by Pekka Koivistoinen and Lea Hyvonen

May 1980, xiv + 290 pp. £17.60 (UK only)/\$40.50 0.12.417050.1

Recent years have seen a rapid increase in the volume of basic and applied research carried out into carbohydrate sweeteners. This has led to the development of many new applications, on the technological side, while much more information concerning the health and safety aspects has also become available. The dominant role of sucrose as a sweetener has not yet been challenged but research is now expected to lead to the development of alternative means of sweetening foods. This important book will be required reading by professionals in the broad area of food science and other related fields. It is, in short, the most comprehensive and thorough source of information currently available about modern applications of sweeteners in food technology.

Advances in Ecological Research Volume 11

edited by A. Macfadyen

April/May 1980, xii + 430 pp. £25.00 (UK only)/\$57.50 0.12.013911.1

The subject matter of the articles in this series ranges over the whole field of theoretical and applied ecology, aiming to present the knowledge derived from primary research to a wide audience. The original papers are all reviews, bringing together the work of many researchers from different parts of the globe and offering previously inaccessible material. In this volume there is an emphasis on the animal kingdom, especially birds, mellanic insects and leafhoppers, while two articles on heavy metals and agricultural ecosystems stress ecosystem studies. This synthesis of recent research is relevant to many theoretical topics, such as evolution, predator-prey relations, island biogeography and species diversity. It will also stimulate and inform readers concerned with environmental management, from the experienced ecologist and agriculturist to the student, the layman and the politician.

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Research Techniques in Nondestructive Testing Volume 4

edited by R.S. Sharpe

April/May 1980, x + 514 pp. £30.40 (UK only)/\$70.00 0.12.639045.1

In this volume, papers on ultrasonic attenuation, ultrasonic scatter, vibrational analysis and neutron scatter have been introduced; these are indicative of the growing interest in the extending scope and potential of nondestructive testing towards monitoring total microstructural uniformity. Advanced signal processing procedures and more computer and microprocessor control also feature increasingly; this marks a significant step forward from the empirical technology on which non-destructive testing has for so long been based. The series also serves to provide an important communications link between those directly involved with the design and use of nondestructive testing procedures in industry, and those in parallel fields of academic research.

Fibrous Proteins: Scientific, Industrial and Medical Aspects Volume 2

edited by D.A.D. Parry and L.K. Creamer

April/May 1980, xvi + 258 pp. £13.00 (UK only)/\$30.00 0.12.545702.2

Unlike volume one, which is essentially a reference work, this second book explores new areas of research and significantly contributes to the furthering of knowledge in this field. Emphasis has been laid on those areas of greatest current interest and which show the most potential for future development. The scientific, industrial and medical aspects of each of the following four subject areas are considered: muscle and meat, collagen and leather, keratin and wool and finally, 'other fibrous proteins' such as elastin, fibrinogen and silk. Although scientists working with different fibrous protein systems employ similar techniques, these topics are usually considered separately. Hence, this volume provides a rare opportunity for research workers not only to obtain up-to-date information on advances in their own field, but also to gain a valuable insight into development in related fields.

Myriapod Biology

edited by Marina Camatini

April 1980, xviii + 448 pp. £28.80 (UK only)/\$66.50 0.12.155750.2

Central though the myriapods are to an understanding of Arthropoda in general, most studies have neglected this fascinating group of invertebrates. The material presented here provides not only a review of the evidence recently achieved with a newer, functional approach, but also breaks new ground in its orientation towards the origin and evolution of the group, both as a separate entity and in relation to the arthropods. It is, moreover, the first volume to condense major topics into separate sections, dividing the presentations among themes such as cytogenetics, functional anatomy and embryology, ecology and biogeography, and endocrinology and life cycle. All sections place emphasis on the evolution of Myriapoda. The last section of the book is devoted to arthropodan evolution in general, and illuminates the stimulating effect exerted by interrelationships and the evolutionary history of these invertebrates.

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edited by Gisele M. Hodges and Richard C. Hallowes

Volume 2 April 1980, x + 316 pp. £24.80 (UK only)/\$57.50 0.12.351002.3

The scanning electron microscope is a most versatile instrument for examining and analysing the microstructure of solid objects. It has proved to be of great value in studies of structure-function relationships in biological tissues as it can provide direct information on the morphology of cell surfaces in normal, pathological and experimentally induced situations. Although scanning electron microscopy is becoming a routine method in biomedicine, until now there has been no publication dedicated to a discussion of its applications. The purpose of this open-ended series is, then, to review and evaluate these applications integrating current knowledge of structure, composition and function into a comprehensive framework.

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Language Production Volume 1

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This and the following volume will bring together all the important research on language production from the fields of adult and developmental psychology, diachronic and synchronic linguistics, phonetics, sociology, neurology and artificial intelligence. Volume 1 focuses on adult speech. A survey of research methods introduces the subsequent reviews which cover the social setting of talk, the psychological processes involved in planning and organizing utterances and the neuromuscular processes that give rise to articulation. Methodologies and materials that apply to the central issues in speech production are in many cases brought to bear for the first time. Traditional research methods are also represented in chapters on aphasia, the significance of hesitations and slips of the tongue, and on motor control of articulation. A final chapter summarizes the implications for a psychological model of speech production.

Royal Society of Medicine International Congress and Symposium Series

Human Health and Environmental Toxicants*

edited by C. Wood

April 1980, xii + 210 pp. £15.60 (UK only)/\$36.00

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This volume presents the proceedings of a conference held on 14-16 May 1979. The subject of this meeting is one which is currently of great concern to both doctors and industrialists, namely, the potential health hazards of modern technology. As Lord Smith of Marlow said in his introductory address "... not only is it extremely difficult to identify the main areas of significant risk; it is difficult to define what constitutes a risk, and still more difficult to say what is an acceptable level of risk. There is a certain balance between risks and benefits, within which it is not only wrong to ignore hazard but also wrong to exclude major benefits to mankind through unreasonable exaggerations of hazards." It was the development and maintenance of this balance, within the context of society as a whole, around which much of the discussion at the meeting centred.

Annals of Botany Supplement No.1

Aspects of the Structure, Cytochemistry and Germination of the Pollen of Rye (*Secale cereale* L.)

J. Heslop-Harrison

January 1980, 48 pp. 18 Plates, £7.80 (UK only)/\$18.00 0.12.344950.2

Despite the importance of the grasses in the human diet, many basic features of their biology remain to be investigated. This is true for aspects of the reproductive physiology of the group, and notably for those connected with pollen, the pollen-stigma interaction and pollen-tube growth. This memoir reports some of the results of recent work on the structure and germination of the pollen of rye and other grass species. The treatment itself is experimental in nature as it combines something of the character of both research paper and review. This is the format envisaged in launching this new series of supplements to *Annals of Botany*. The supplements offer authors the opportunity not only to report new findings, but also to relate them to those of other workers in more detail than is usually possible within the confines of the normal research paper.

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Volume 2: February 1980, 328 pp. £30.60 (UK only)/\$52.50 0.12.058502.2

This two-volume work gathers together the diverse information presently available on spontaneous animal models of human disease. In addition to providing a comprehensive review of existing models, the book presents many previously unpublished new models. A conscious attempt is made to point out the similarities and dissimilarities of each model to its human counterpart. The authors discuss many long proffered models with regard to their limitations in biomedical science.

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James R. Holton

November 1979, 416 pp. £12.80 (UK only)/\$22.00 0.12.354360.6

The standard textbook in its area, *An Introduction to Dynamic Meteorology* has been considerably expanded and updated for this Second Edition. Holton approaches dynamic meteorology in a logical fashion as a coherent subject with a central unifying concept, namely, the quasi-geostrophic system. Quasi-geostrophic theory is used to develop the principles of diagnostic analysis, numerical forecasting, baroclinic wave theory, energy transformations, and the theory of general circulation. Throughout the text emphasis is on physical principles rather than mathematical elegance. Each chapter contains numerous problems and examples. Answers are included to selected problems. The text is addressed primarily to senior and beginning graduate level students in meteorology, but any student of the physical sciences who has mastered introductory-level physics and calculus will find the material accessible.

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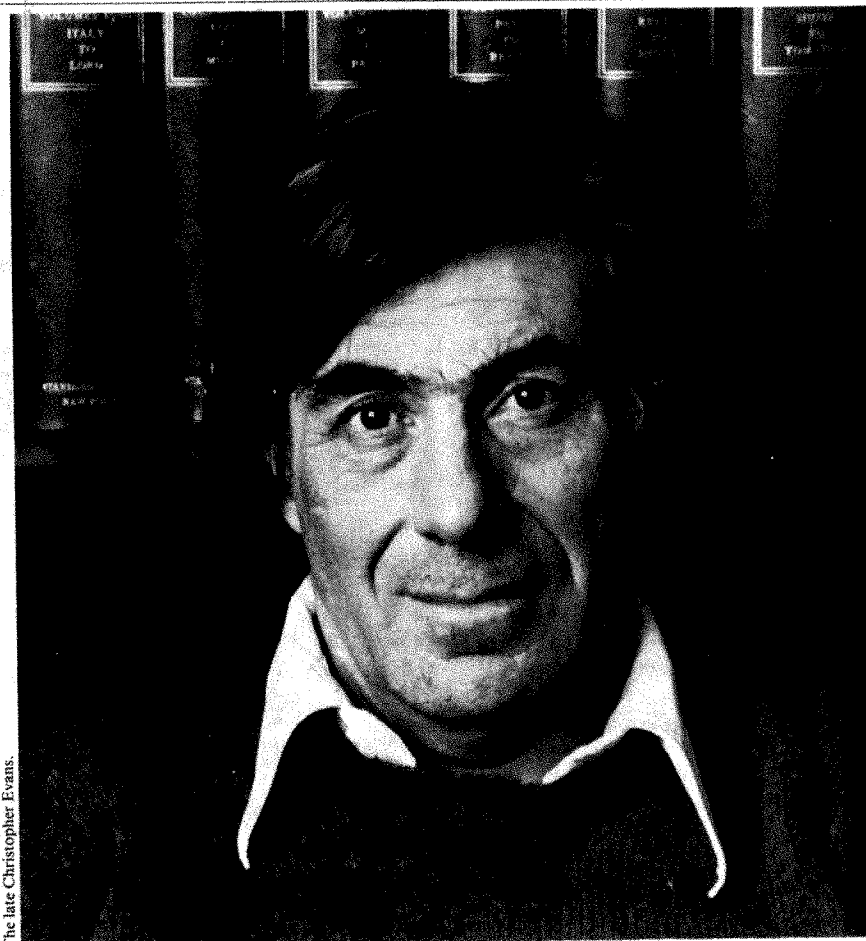
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future for computing, but without the invention of the silicon chip their visions could never have been realized, and that invention, as Dr Evans points out, could never have been foreseen. It transformed the cost, speed and reliability of computers, and made it practical to multiply the components almost without limit.

Most of *The Mighty Micro* is devoted to the future, and is speculative rather than closely argued. Dr Evans relies heavily on analogies, many of them striking. To give the reader an idea of what the exponential growth in computers portends, he points out that a piece of paper folded on itself 50 times would stretch beyond the orbit of Mars, a result that few would guess without calculation. The analogy may be better than he realizes: just as there are practical limits to such exponential growth in the thickness of a piece of paper, so there are invariably factors that set limits to the continuing exponential growth of a new technology.

Perhaps the most obvious such factor is the provision of the sort of software Dr Evans envisages. He is surely being unduly sanguine when he writes "There is no suggestion that before substantial improvement [in machine intelligence] can take place, any new principles must be invoked or new discoveries made". It is true that advances in artificial intelligence have over the last ten years thrown some light on how the human brain is likely to execute certain tasks, but the mirage of actually using intelligent programs to do something useful constantly recedes from us and there is still only a handful of applications. One reason is that very few general principles have been discovered, and it is necessary to work out *ab initio* the structure of each new intelligent task before attempting to program a computer to execute it. Hence, computers have so far proved to be an effective substitute for the human brain only in tasks, such as mass spectrometry and chess, the structure of which has been made explicit and which necessitate a search through a vast number of alternative possibilities. Where, as in economic decision taking, we have little understanding of the task, computers cannot carry it out unaided, and are probably more of a hindrance than a help to our own thinking if used as a tool, since the spurious authority of computer forecasts may make us forget the fallibility of the assumptions on which the forecasts are based. As for Dr Evans' claim that we will shortly be able to design a machine that is more intelligent than ourselves and which can in turn design machines more intelligent than itself, we have at the moment no inkling of how to set about such a problem; nor is there any evidence to suggest that it would ever be possible. There remains, then, an astounding gap between the prognostications of some practitioners of AI and the performance of existing programs, and there is no sign of



The late Christopher Evans.

Courtesy of Victor Gollancz Ltd.

the impending discovery of a software equivalent of the silicon chip that will open the way to dramatic advances in the subject.

Although Dr Evans may have compressed the time scale, computers will inevitably change our living conditions; but his enthusiasm for the prospect will not be shared by everyone. He appears to think that the extra leisure time left when computers are performing most of both the important and the menial jobs can be filled by playing games against them: but people like to feel they are doing something useful and a life devoted to such games would be a hell of boredom. The Greeks, provided with leisure by slave labour, occupied themselves with making war on their neighbours, parochial politics and speculations about the nature of the Universe. We can no longer fill our time by making war, and if computers were ever able to take political decisions more sensibly than ourselves and to undertake advanced scientific work, it would come to seem pointless for us to engage in these activities. It is perhaps fortunate that in the lifetime of anyone now living we are unlikely to design our own intellectual successors, and hence render ourselves redundant. □

Stuart Sutherland is Director of the Centre for Research on Perception and Cognition, University of Sussex, Brighton, UK.

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Illusions of pedigree

J.D. Mollon

Seeing: Illusion, Brain and Mind. By J.P. Frisby. Pp.160. (Oxford University Press: Oxford, 1979.) £6.95.

THOSE who teach experimental psychology will instantly recognize the pedigree of this handsome colt: it is sired by Richard Gregory out of Lindsay and Norman. It lacks the turn of speed of its parents but proves an excellent stayer on soft ground, is usually reliable over hurdles and should bring rewards to its backers.

Professor Frisby is to be congratulated on an engaging and remarkably lucid introduction to the problems of visual perception. His declared purpose is to combine the psychological, physiological and computational approaches to perception. He has a good judgement of the important issues. Topics covered in detail include feature detection, figure-ground differentiation, the anatomy and physiology of the visual cortex, object recognition, the computation of lightness, and binocular stereopsis. The concept of spatial frequency and the contrast sensitivity function are deftly introduced for the novice reader. The book is sumptuously and skilfully illustrated and the figures allow readers to experience for themselves many illusions and perceptual after-effects; indeed the author recounts in his preface how he originally set out to produce a collection of illusions for the general reader but, like a novelist taken over by his characters, was soon led to provide the intellectual background. Two-colour anaglyphs, many of them original, demonstrate phenomena of binocular vision. The major omission is a discussion of colour vision; this is odd, even inexcusable, given the lavish facilities available for colour illustration.

The exposition is one of almost unfaltering clarity and this is perhaps the most impressive quality of the book. If Frisby has any fault it is that of spelling points out too much — a virtue in a lecturer but a vice in an author; thus he devotes no less than ten, similar, figures to introducing the concept of a corner-detector.

In elaborating the computational approach to visual perception, Frisby acknowledges an explicit debt to D. Marr "whose writings are for me the work of genius". He discusses in detail, for example, Marr's theory of how the retina computes lightness, that is, discovers the reflectances of surfaces that are unevenly illuminated: bipolar cells detect edges by a centre-surround differencing operation; a threshold is applied to the resulting signal; and then (the "deconvolution" stage) lateral facilitation within one class of

ganglion cells causes all cells corresponding to points within a closed boundary to take on the lightness-signal indicated by the signal at the edge. Now, Marr's histological reification of this algorithm is, as Frisby says, "controversial" (to say the least), but there is a more general difficulty. The whole of Chapter 1 was devoted to disabusing the novice reader of a belief in the 'inner screen' theory of perception (the view sometimes called the Gestaltist fallacy); if our novice reader has taken that chapter to heart, then, when he reads Frisby's account of the computation of lightness, he will want to know why the visual system goes to such trouble to produce a picture of the scene in which lightnesses are redundantly represented in the interiors of homogeneous areas, why, that is, the deconvolution is necessary. And he won't be told.

Frisby leaves to a late stage in the book the details of retinal anatomy and physiology. Perhaps it is in this area that he is least sure of himself, for it is here alone

that errors occur in any density: in the course of two pages, the inner and outer synaptic layers are confounded, the pigment epithelium and choroid are labelled "sclera", rods are said to be 500 times more sensitive than cones because they contain rhodopsin, the fovea is said to contain only cones, an error of 10^3 is made in equating retinal extent to visual angle, and the amphibian mudpuppy (in a remark apparently lifted almost verbatim from Blakemore) is described as a fish. Undergraduates should be directed elsewhere for their retinal physiology.

The contributions of the editor, Henry Hardy, and the art director, David Warner, are so manifest in this book that their work deserves explicit congratulation. *Seeing* will be equally in place on the coffee table and on the first-year reading list. I strongly recommend it. □

J.D. Mollon is a Lecturer in Experimental Psychology at the University of Cambridge, UK.

Flawed search

Sara Champion

In Search of Ancient Astronomies. Edited by E.C. Krupp. Pp.277. (Chatto & Windus: London, 1980.) £8.95.

THIS book arose from a series of lectures given by the contributors in California in 1975, and is, according to its editor, "the first attempt to present systematically to the general reader the main results of archaeo-astronomy to date". It consists of seven chapters, four by the editor and one each by Professor Thom and his son, Dr John Eddy and Dr Anthony Aveni. It is clearly stated in Krupp's introduction that the "pseudoscientific misconceptions" of Erich von Däniken, Velikovsky and the like are to be "dispelled by the reliable, scientific findings of archaeo-astronomy"; it is unfortunate, therefore, that the majority of the chapters show some misuse or misunderstanding of archaeological data and imprecision in the demonstration of astronomical alignments.

The first chapter by Krupp himself is presented as a non-mathematical introduction to practical astronomy. This is necessary for the understanding of subsequent chapters, and is in general comprehensible, though some of the information could have been transmitted with fewer words and greater clarity.

For many readers the work of the Thoms will be the most familiar, and their chapter on stone circles and menhirs follows. Thom's eminence in the field is largely based on the accuracy of his measurements and the precision of his analysis. Recent papers and reviews have, however, cast doubt on some of his figures, with serious

results for his megalithic yard and megalithic calendar (Moir *et al.* *Antiquity* LIV, 37-43, 1980). Misidentification of archaeological monuments has resulted in Thom's describing hut circles and enclosures as stone circles of megalithic type, and some of the lines of foresight suggested for certain sites are shown in the field to be below horizon level, difficult to see without binoculars or blocked by natural features not obvious from maps. Some controversial sites appear in this chapter: the Crucuno Rectangle, for example, described by Thom as "lunar standstill alignments", is claimed by Daniel in *Antiquity* XLIX, 81, 1975, to be an AD eighteenth century folly. For the archaeologist prepared to be convinced, some of Thom's claims appear thin and several statements contentious. To be told that, at Rough Tor in Cornwall, many of the upright stones have fallen and the remaining ones do not adhere closely to the flattened circle design, perhaps because of solifluxion, begs the question — what flattened circle design is there left to be measured. Rules about the use of megalithic yards are stated, only for exceptions to be made immediately: "the sides of these triangles all had to be integers in megalithic yards", followed by "the builders discarded the rule that all radii must be integers".

The third chapter, by Krupp, deals with the work by archaeo-astronomers and others on the site of Stonehenge, several of whose papers have appeared in *Nature*. In general the treatment is descriptive rather than critical, which may be misleading for the general reader. Analysis of Hoyle's claim that Stonehenge was an eclipse predictor has been published by Moir (*Antiquity* LIII, 124-128, 1979); he points

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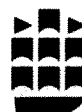
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M Introduction to NUCLEAR REACTIONS

G. R. SATCHLER

— Research staff member,
Oak Ridge National
Laboratory, Tennessee.

*Spring 1980; £25; 336pp;
ISBN 0 333 25907 6.*

Contents include — Some Background; Introduction to Nuclear Reactions; Elementary Scattering Theory; Models of Nuclear Reactions.

This book presents a summary of our present understanding of the Scattering and Reactions that occur in collisions between nuclei and is an introduction to the theoretical formalisms used to describe them. It is aimed primarily at readers in the undergraduate and beginning graduate student level but many others will also find it useful. The specialist from another field will find that this book is excellent background reading and the established nuclear physicist will find reading it to be helpful in refreshing the memory about areas in which he or she is not currently working.



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out the problems of refraction, visibility, and variability in moonrise and moonset positions due to declination and perturbation which would affect the use of the site in this way. The same criticisms can be applied to Thom's work where it involves lunar observations. Again, the misuse of archaeology undermines attempts to prove alignments: Krupp's use of Figsbury Ring, at least 1,000 years later in date than Stonehenge, as a foresight for the southern major standstill moonrise, is inexcusable.

Eddy's chapter on North America is the only one in the book where a sensible caution appears. His balanced view of others' work is exemplified by his regard for the archaeology as equally important as the astronomy in the analysis of the Chaco Canyon structures. His own work on the Indian medicine wheels is a model of careful and logical procedure. The same cannot be said of Aveni's chapter on

Mesoamerica, where sites a millennium and several hundred miles apart are grouped on the basis of their similar orientation. The use in this chapter of words like "nearly", "close to" and "approximate" when describing alignments destroys their credibility. The claimed alignment of Teotihuacán on the setting of the Pleiades turns out to be "within 1 degree" of this event, a difference representing a sizeable slice of the horizon. Misunderstanding of archaeological data in this chapter results in Aveni using Flannery and Marcus' application of central place theory to Mayan settlements as evidence of geometrical and possibly astronomical location of these sites.

Krupp's chapter on Egyptian astronomy, mainly descriptive of previous work, is followed by his attempt to debunk von Däniken and the rest. His handling of the leyline controversy and the Glaston-

bury Zodiac lacks conviction, since he frequently ignores the most obvious line of attack; for example, he reproduces without comment the notorious leyline that runs from Stonehenge (third millennium BC) through Old Sarum (sixth century BC) to Salisbury Cathedral (AD twelfth century).

With the exception of Eddy's chapter, the book fails on its own terms, for it presents to the reader a picture based, in many cases, on misunderstood archaeological evidence and selective or imprecise numerical and astronomical data. The non-expert may unfortunately be persuaded by the jaunty air and punning subheadings (e.g. "A Serious Mystery" for a section on Sirius) into believing it all. □

Sara Champion is Hartley Fellow of the University of Southampton, UK, in the Department of Archaeology.

FRS extraordinary

J. Z. Fullmer

Benjamin Thompson, Count Rumford. By Sanborn C. Brown. Pp.576. (MIT Press: Cambridge, Massachusetts, and London, UK, 1979.) \$19.95, £12.40.

BENJAMIN Thompson, born in March 1753 in Woburn, Massachusetts, died Benjamin Thompson, Count Rumford, in August 1814, in Auteuil, France. By almost any criteria his biography should make a rattling good yarn, for his adventurous life had episodes of intrigue, of mystery and of passion. He promoted large scale social experimentation and technological innovation; he interpreted his scientific experiments in ways that propelled him into controversy with the conservative members of the scientific community. When young and a volunteer member of the American army he functioned as a Royalist spy. In the spring of 1776, he fled to England, where he promptly ingratiated himself with influential British government officials. In 1783 he went to the Continent, becoming an agent for the British in Munich (or, perhaps, a double agent — the record is cloudy). Subsequently he shuttled back and forth between England, Ireland and Europe with no apparent regularity or design, his movements dictated by opportunity. He acknowledged two illegitimate children, one in Munich, the other in Paris, and sired one legitimate child, Sarah, born in Massachusetts. Assured that his abandoned American wife was truly dead, he married Jeanette Lavoisier (*née* Paulze), Antoine Lavoisier's widow, from whom he was soon divorced after a series of spectacular and public brawls. Moreover, during long

periods in England and on the Continent he associated closely with well-placed reputed homosexuals, furthering his own career through these connections.

He managed to be close to centres of power in the government circles to which he propelled himself, and in the international scientific community. In 1780 he was elected Fellow of the Royal Society — he was 27 — after having attracted favourable notice for his very long paper on the nature of heat. For nearly a decade he earned the attention of Karl Theodore, the Elector of Bavaria, to whom Rumford owed his honorary title. The scientifically conservative Napoleon showered attention on him, and in England Sir Joseph Banks, PRS, abetted some of his plans. Rumford published a large number of papers: on the nature of heat

and of light; on the best designs for stoves; on ways to feed the poor; on the efficient use of fuels in kitchens and fireplaces; on the boring of cannon; and on a pot for brewing coffee. He laid out the English Gardens in Munich; he devised plans to reduce beggary in Bavaria while feeding and clothing the army; he promoted the erection of a technological museum that offered visitors 'hands-on' experiences; he attempted to educate artisans in the factory ways of the industrial revolution; together with Banks he founded the justly famed Royal Institution of Great Britain.

Yet even this extensive listing of his promotional efforts does not exhaust them. While still alive Rumford proffered sums of money to England and America, endowing the prizes known as the Rumford Medals of the Royal Society and of the

Demonstration lecture by Thomas Young and Humphry Davy, with Count Rumford watching.



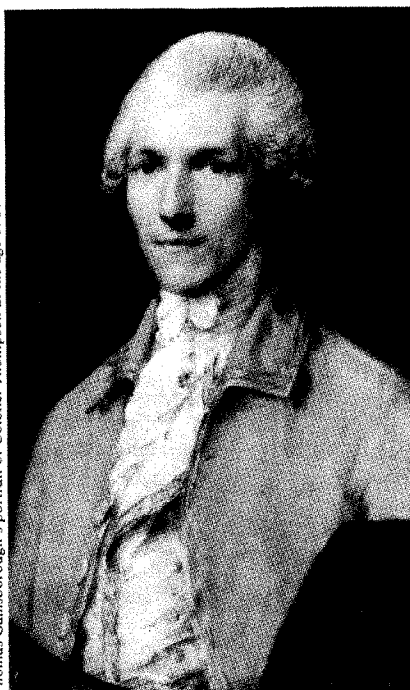
American Academy of Sciences. Instructions in his will established from the residue of his estate the "Rumford Professor of the Physical and Mathematical Sciences as applied to the useful Arts" at Harvard. These eponymous memorials furthered his imprint on the scientific community. Perhaps Rumford was not unique, for adventurers of all sorts abounded in revolutionary times. Moreover, the history of technology provides us with occasional examples of scoundrels — but Rumford must be unique in the degree of his rascality. He appears to have been a man devoid of real allegiances, clever enough to exploit the political turmoil and the scientific conceptual turbulence of his times for his own good.

Sanborn C. Brown, Emeritus Professor of Physics at MIT, presents the fruits of nearly 40 years of search for the original documents relating to Rumford's career. For these efforts every subsequent scholar will be grateful. In 1962 Brown wrote *Count Rumford Physicist Extraordinary*, one of the publications in the Science Study Series, which related in a chronological way what he had learned of Rumford's life. Brown's new biography expands upon that earlier book without deviating from its patiently explanatory tone and linear structure.

Brown, of course, has not been alone in paying attention to Rumford's life and accomplishments. Many historians of science, historians of technology and sociologists of science have examined portions of Rumford's activities, and it is to the neglect of this informing and analytical literature that Brown's biography owes its greatest weakness. Examples are not difficult to come by. Robert Fox in *The Caloric Theory of Gases* (Oxford University Press, 1971) has examined Rumford's work on the nature of heat, and placed Rumford's ideas within their contemporary milieu. Although Brown cited Fox's work, he did not use it to inform his own discussion. Morris Berman has analysed the early days of the Royal Institution in *Social Change and Scientific Organization: The Royal Institution, 1799–1844* (Cornell University Press, 1978). While Berman's general interpretation and analysis is the subject of considerable debate, much of what he has said is both useful and informative to the biography of Rumford. Brown appears to have been unaware of Berman's contributions.

Reading the biography of any practising scientist (even a part-time scientist, as Rumford assuredly was) raises the question: what is the most important aspect of such biography? To be sure, the biographer owes his subject as accurate a chronicle of the chief events in the life as can be achieved. Brown has provided that for Rumford. But the biographer of a scientist also owes his reader the benefits of his analysis of the subject's endeavours, of his achievements as seen by his

contemporaries, of his failures as judged by his contemporaries, and an assessment of where the subject fits into the histories of science and of technology. While Brown discusses Rumford's work on the nature of heat chronologically, he has not truly shown what motivated Thompson's extensive experiments. What is missing is the deep analysis which a historical study requires. When Brown discusses Thompson's social reforms he chronicles the major efforts, but he fails to show whether Thompson regarded his projects as intellectual solutions to complicated puzzles, as palliatives to grievous human needs or as a means for currying personal favour for himself. Above all, Brown does not appear to have realized how much of an outsider Count Rumford was; the narrative suggests that Rumford functioned at his best only when he enjoyed a perverse, self-engendered alienation. Perhaps, in this instance, the abiding question should have been "What made Rumford run?"



Thomas Gainsborough's portrait of Colonel Thompson at the age of 30.

Physicists publishing research findings must do more than offer raw data. Generally, that data has to be placed within a larger conceptual framework. The historian and the biographer have the same obligation. To view a man as a two-dimensional cardboard cut-out (no matter how colourful), jerked into spasmodic action by unseen wires and strings against a flat backdrop, impoverishes our understanding of him. It is on this score that Brown's biography of Rumford is most disappointing. Brown's biography is not the definitive life of Rumford, but it does offer a brave factual beginning on which subsequent analysis must rely. □

Mrs. Fullmer is Professor of the History of Science at Ohio State University, Columbus, Ohio.

Of special concern

Austen Albu

Science and Technology: A Five-Year Outlook. By the National Academy of Sciences. Pp.544. (Freeman: San Francisco and Oxford, 1980.) Hardback £8.90; paperback £5.40.

IN 1977 the United States Congress legislated for a periodical Five-Year Outlook on Science and Technology. The responsibility for this work was eventually transferred to the National Science Foundation. In 1978 the Foundation asked the National Academy of Sciences to prepare a report describing the current state of significant research areas and pointing out those areas which would be of special concern within the five year period. A review procedure by members of a number of other bodies was established. Those involved included 50 contributors, 20 editorial consultants, and 170 reviewers and additional contributors. Although the resulting report is by no means comprehensive and only intended as a preliminary study, it covers a number of fields in encyclopaedic fashion and one can only express admiring astonishment that it should have been produced by March 1979.

British Members of Parliament have long envied the resources available to their American colleagues and, for all their growing interest in science and technology during the past few years, they would never have succeeded in commissioning a report on this scale. Written in a straightforward style, not over-popularized, it offers descriptions of recent and likely future developments in such fields as geology, biology and biochemistry, physics, computers, energy, and US demography and health. Some of the subjects are politically sensitive and tend to be dealt with in a rather didactic manner; but on the whole the problems requiring research in the near future seem to have been set out fairly. Of course neither the average congressman nor this reviewer is capable of judging the scientific quality of these descriptions and proposals, and the problem of choice of priorities remains as difficult as ever. I am reminded of a remark of my son's when, in his twenties, he was facing a choice in his life on which he had consulted his liberal parents: "Just like my parents, tell me all the pros and cons and leave the decision to me".

Two criticisms can be made of this report: there are no price tags attached to any proposals for the future, without which serious consideration of alternatives by a legislative body is impossible. Perhaps equally serious is the lack of any reference to Defence R&D, the cost of which overshadows all the rest and which itself

contributes indirectly to so many other fields.

The problems now facing American scientists and engineers, though vastly greater in scale, are common in advanced industrial countries. There has been a collapse of the academic boom of the 50s and 60s. Average annual growth at constant prices of funding for academic R&D from 1953 to 1960 was 12%; from 1960 to 1964, 14%; from 1968 to 1974, zero; and from 1974 to 1978, about 4%. Despite increased numbers of graduate students, doctorates in science and engineering are declining. Total support for basic academic research declined by 8% between 1968 and 1976 and federal support by 10%.

Naturally the overwhelming number of developments reported are concerned with

practical and, in some cases, urgent problems: energy, material resources and health. Their solution is difficult enough in scientific terms but is increasingly hindered by social and political considerations. These are not only panic reactions as in the case of genetic manipulation or nuclear energy after Harrisburg but arise out of growing concern with environmental questions, leading to regulatory procedures. No doubt there is some special pleading here. Even the possibility of expanding the use of coal is hindered by the opposition of agrarian states to its mining in their territories and by fear of the atmospheric effects of its use in power stations. For this reason conservation is priority number one in the energy field. Of course vested interests are involved and indications of these can be detected in some

of the reports. Political decisions can also distort the allocation of R&D resources, tactfully referred to in an account of the examination by "a new leadership" of the "enormous effort" of Nixon's "War on Cancer" in order to achieve a more balanced programme.

Many readers of this fascinating book must be left with two impressions: the enormous area remaining for scientific R&D to deal with the world's material problems and search for knowledge; and, in a divided world, the considerable international cooperation here reported and the need and scope for its further enlargement. □

Austen Albu was a Member of Parliament from 1948 to 1974, and a Visiting Research Fellow of the University of Sussex Science Policy Unit, Brighton, UK, from 1975 to 1979.

Bringing Man poetically to life

Yves Coppens

The People of the Lake: Man, His Origins, Nature and Future. By Richard E. Leakey and Roger Lewin. Pp.223. (Collins: London; Doubleday: New York, 1979.) £6.50.

People of the Lake is a kind of essay on Man. Pleasantly readable, it puts forward in a little over 200 pages an overall view of our species in both a historical and comparative context which is thoroughly satisfying for anyone with a naturalist's turn of mind. Although the reader may regret a certain lack of rigour in the comparative approach, as too great an emphasis is placed on seeking out good examples to back up the argument at the expense of a critical attitude towards similarities and differences, the attempt is nevertheless an extremely interesting one, and one worthy of being encouraged.

Divided into 11 chapters, the book is in fact made up of two sections of almost equal length; the first of these, comprising the first five chapters and part of the eleventh, is devoted to palaeontology, the origin and evolution of Man, from the first primates of 70 Myr ago to *Homo sapiens sapiens*; the second (Chapters 6, 7, 8, 9, 10 and the remainder of Chapter 11) concerns itself with ethology, human behaviour and the search within the animal kingdom for Man's origins and the direction of his evolution. Given the training and special interests of the authors, this second part is more an essay in prehistoric ethology: just like chimpanzees, gorillas, baboons or geladas, contemporary Man is used in an attempt to reconstruct the lifestyle, psychology and behaviour of ancient Man. In spite of what

might be inferred from the sub-title, neither the future of mankind, nor even his present condition, seem to be the writers' principal concern.

The point of departure — hence the title — is the palaeontological research carried out by Richard Leakey over the past 12 years on the shores of Lake Turkana in Kenya. In 1967, Richard Leakey, together with Francis Clark Howell and myself — and initially Louis Leakey, his father, and Camille Arambourg — played a part in setting up the important international expedition along the Omo River in Ethiopia, to the north of Lake Turkana itself; and it was during one of the many flights that had to be made back and forth between Nairobi and his camp on the Omo that he noticed the sedimentary formations outcropping on the eastern banks of the lake. He decided there and then to investigate, and since 1968 a dozen or so separate expeditions have provided an extraordinary collection of human fossils, their age ranging from more than 4 Myr to less than 1 Myr and including half a dozen skulls in an excellent state of preservation, highlighted by the famous ER-KNM 406 (*Australopithecus boisei*), 1470 (*Homo habilis*) and 3733 (*Homo erectus*). These expeditions, known as the Koobi Fora Project, make up one of the four large-scale international projects under way in the Rift Valley of East Africa and concerned with the problems surrounding the origin of Man. The other three are the Olduvai expedition (more recently Olduvai-Laetoli), originating with Louis Leakey as long ago as 1931 and under the direction initially of Louis Leakey, then of Louis and Mary Leakey and, since Louis' death in 1972, of Mary Leakey alone; the international Omo expedition for which Francis Clark Howell and myself were responsible over eight further seasons after the departure of Richard Leakey in 1968; and the Afar international expedition, which began work in 1972 and has since been under the supervision of Maurice

Taieb, Donald Johanson and myself. The People of the Lake themselves, of course, are these fossil men of about 2 Myr ago (*Australopithecus boisei* and *Homo habilis*), whom the authors bring poetically to life again as early as the first chapter of the book, in a reconstructed landscape on the banks of a palaeolithic Lake Turkana.

The palaeontological résumé provided is rather conventional but not without interest. It traces the links between Cretaceous and Quaternary forms, the change from a nocturnal to diurnal life (and the consequent improvement in eyesight), and, much nearer in time to ourselves, the straightening up of the body and its probable successive implications — the freeing of the hands, work, development of the sweat glands, loss of hair and the appearance of a protective pigmentation for the body. Considerable passages of the book are devoted to this possible ancestor of Man, the quadruped *Ramapithecus*, and his erect descendant the *Australopithecus*, and to the irritating gap of 4 Myr that separates them.

The ethological aspects examined in the second section of the book represent in many cases interesting approaches to problems which are not usually dealt with in the context of prehistory. They all arrive at the same fundamental conclusion: that Man is as he is because at the time of his origin he shared his food with the other people in his group — the development of his intelligence (and of his language, which is no more than a manifestation of his intelligence), the extraordinary transformation of his technology, his inclination for reciprocal altruism and for family life, even his obsession with sex — are the fruits of his cooperative life and of his mixed economy based on the male's hunting and the female's gathering: everyone is in part dependent on others. With this idea established as their foundation, the authors go on to debate a wide range of problems raised, rightly or wrongly, by our times, or by other writers whose success has

moulded our view of our times: the hypothesis of the essential role of hunting in the evolution of Man, the counter-hypothesis of the preponderant role of gathering, the problem of Women's Liberation, the still unsettled question of the origin of aggression and of the phenomenon of war, and so on.

It is unfortunate that these topics are touched on only rather superficially, though it could scarcely be otherwise in such a limited space; from this point of view it must be admitted that the reader's appetite is never entirely satisfied: he is left not wholly convinced by an argument too fragmentary in its evidence. Nonetheless, the very fact that some additional light has been thrown on all these various problems by authors possessing zoological, ethological, prehistorical, palaeontological and ethnological knowledge, obviously deserves a salute. Indeed, a second salute, as I have already stressed the importance of a comparative approach in the introduction to the French edition of the present authors' earlier work *Origins* (for a review, see *Nature* 270, 108; 1977).

Nevertheless, I cannot agree with every statement made in this book. It may be instructive to look at some of the points of disagreement. On page 73, the *Australopithecus* is introduced to us as a possible herbivore although there is little likelihood that it can actually have been one; he was in fact not sufficiently far removed from Man to have had a fundamentally different digestive apparatus. And Man is incapable of digesting cellulose.

Between 1955 and 1960, Johannes Hürzeler, in connection with his study of the *Oreopithecus*, pointed out the great antiquity of the phenomenon of bipedality in nature. A demonstration (page 70) of the existence of bipedality only 4 Myr ago is not therefore going to come as much of a surprise to vertebrate palaeontologists, who for the past twenty years have been expecting to uncover its existence in the Miocene.

The notion (page 131) that the brain was among the first organs to take on human form, rather than the contrary suggestion, has long prevailed; hence, the celebrated Piltdown hoax of a fossil man with a modern brain and pongid teeth. And when authentic and extremely ancient fossils, such as the first Taung *Australopithecus* skull, have been brought to light, it has been quickly established that the brain, although of modest development, was different from that of the great apes.

One should not confuse (page 92) written knowledge with the knowledge of nature that can be acquired in the field; any farm worker in any Western country, living in close contact with his surroundings and often hunting game there, would surprise Richard Leakey and Roger Lewin with the quality and diversity of his knowledge of the fauna and flora, even of the very rocks, of the countryside about him.

How is it possible (page 126), in a work

which (rightly) attempts to demonstrate the precarious nature of the borderline between the apes and Man, in respect of the use (and manufacture) of tools and the notion of natural facing elements (the rain dance of the chimpanzees, for instance), to come out with a statement as specific as "we suggest that it was primitive *Homo* . . . who made tools and, furthermore, that it was only *Homo* who did so"? Why is it not possible that the *Australopithecines* may have made a beginning which Man inherited and then developed brilliantly? Linking the tool so dogmatically to the genus *Homo*, the authors throw the definition of the genus into confusion — a definition which should remain strictly biological.

May I finally be permitted to give vent to my irritation at the habit, not at all international in spirit, of expressing all measurements in inches, feet, yards, miles and square miles; in 1980 we are all citizens of the world!

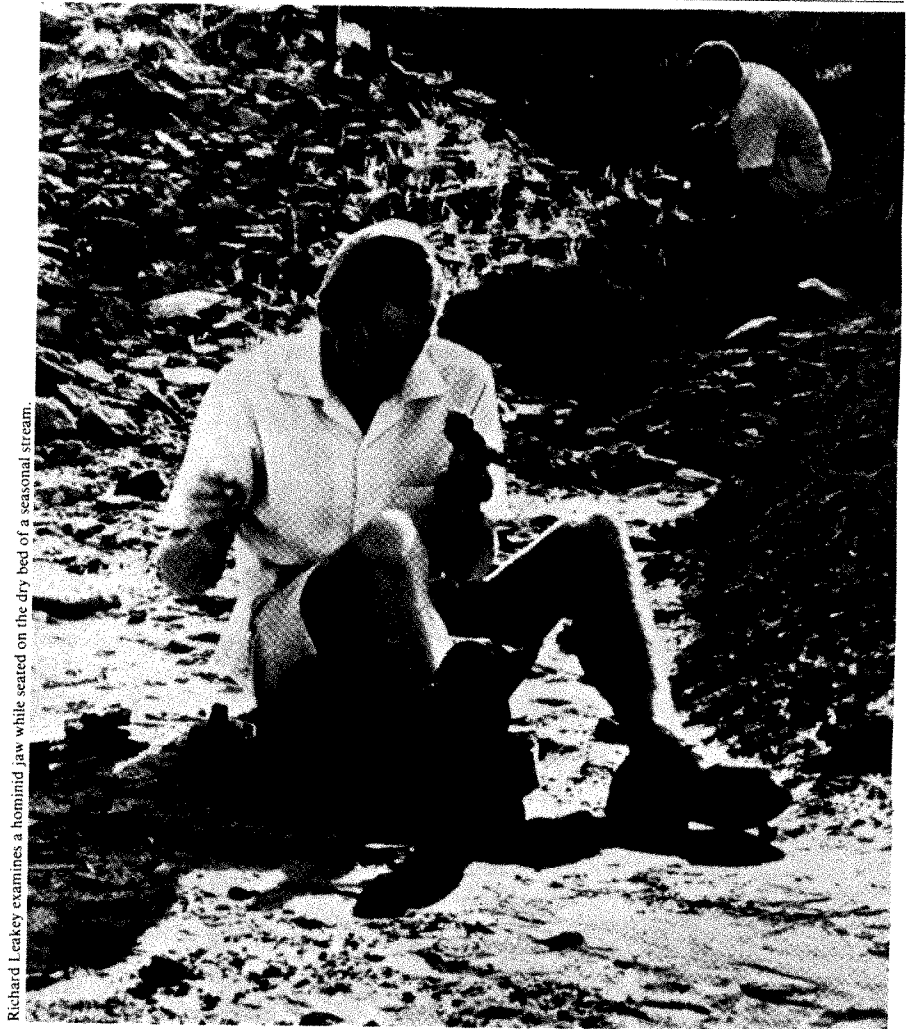
These differences are, it need hardly be said, extremely minor in the context of an overall impression of a highly intelligent work, which has the great merit of linking natural sciences and the humanities.

Let me now turn from these basic issues to make a few remarks on form. As I pointed out at the beginning of this analysis, this is certainly an admirably

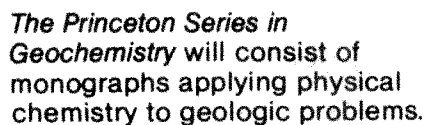
readable book. It is made so by its style, and by an approach which, though scientific, is always deductive. It is enlivened by the use of entertaining asides, such as anecdotes like those describing how Tom Gray and Donald Johanson discovered Lucy, the context in which Ralph Holloway studied his endocranial casts, or the anxious cleaning of skull 3733 by Alan Walker; and, not least, by the interrogative way which they sometimes use to outline the problems better; the questions asked are precisely those the reader is likely to be asking himself.

We learn by this book that Man is very ancient; his origins, both morphological and behavioural, have deep roots in the animal kingdom. It is by studying this kingdom and systematically analysing all contemporary human societies that we may come up with the answers to questions about our own origins, the process of our evolution and, consequently, to some limited extent, about our future. It is only through a better knowledge of our past and of all that can illuminate it that we may come to be able to foresee, and forestall, our destiny. □

Yves Coppens is Co-Director of the Omo and Afar international expeditions, and Deputy Director of the Musée de l'Homme, Paris.



Richard Leakey examines a hominid jaw while seated on the dry bed of a seasonal stream.



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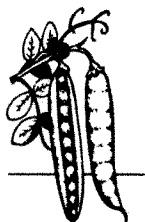
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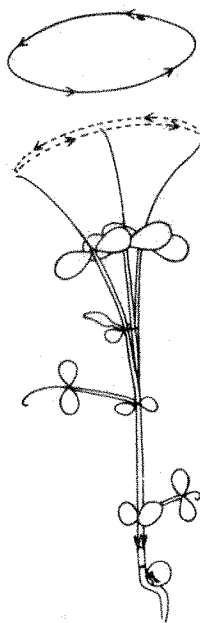
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Oceanographic Institutions — Science Studies the Sea. By P. Limburg. Pp.265. (Elsevier/Nelson Books: New York, 1979.) \$9.95.

THE public image of a scientific laboratory and of the scientists who work in one is highly coloured by what is seen on television and by science fiction. It is therefore very valuable for a book to be published which explores the reality of research institutes and attempts to look at the motivation and day-to-day working of research scientists. By choosing a subject such as oceanography which has grown rapidly during the past three decades, but the roots of which go back to the last century, it is possible to relate the development of the science with the development of the laboratories studying it.

Limburg has written a book that tackles with varying success both the science and

the laboratories. As a potted history of oceanography his first three chapters leave a good deal to be desired. There are inaccuracies (the first word in the acronym ASDIC is "Allied" not "Anti-"), a lack of understanding of some of the technology (the difference between reflection and refraction seismics) and a rather journalistic view of the development of scientific thought (from continental drift through stable continents towards plate tectonics). The historical review is very unbalanced. Two pages are devoted to diving and subsea habitats, whereas deep-sea drilling is limited to 11 lines, and oceanography from space to six. Near-bottom technology scarcely gets a mention nor do the achievements of long-term deployments of instruments in the ocean, which depend crucially on the developments of marine acoustics.

The accounts given of the work in the major (and some minor) oceanographic laboratories in the USA are both informative and interesting, and the unravelling of the complexities of US Government agencies' funding of ocean research is revealing, if perhaps somewhat

ephemeral. As the book proceeds, it becomes clearer and clearer that the author is really concerned with oceanography in the USA and I wonder why this was not made clearer in the title. Oceanography in all other countries is limited to one chapter and appears to include only those laboratories which the author was able to visit. No mention at all is made of oceanographic institutions in the USSR or in Japan. It cannot therefore be said, nor is it claimed in the Introduction, that the book is a comprehensive review of oceanographic institutions. What it does provide, and in this is its success, is a peep in through the doors of some of the laboratories, an insight into the enthusiasm and motivation of some oceanographers both great and small, and an indication of the breadth of the subjects studied.

For a student wishing to start a career in oceanography, especially in the USA, I warmly recommend this book. □

A. S. Laughton, Director of the Institute of Oceanographic Sciences of NERC, has spent his working life in the field of marine geophysics, and has worked closely with several of the major oceanographic institutions in the USA.

The wonder of it all

R.H. Dalitz

Knowledge and Wonder: The Natural World as Man Knows It. Second edition. By Victor F. Weisskopf. Pp.312. (MIT Press: Cambridge, Massachusetts, and London, UK, 1980.) Hardback \$15, £9.30; paperback \$5.90, £3.70.

TODAY we have knowledge of our Universe covering distances from 10^{-16} cm to 10^{+28} cm, and for time intervals from 10^{-24} s to 10^{+17} s. All the phenomena observed within these limits are apparently governed by a very limited number of physical forces. The aim of this book is to lead non-scientists to appreciate the wonder of this situation, first by indicating simply how we know the facts and then by illustrating the wide variety of phenomena governed by these forces. Such an endeavour necessarily involves some oversimplifications, of course. Here the job is done in low key, showing how simple concepts based on the laws governing these forces can lead to an understanding of this strikingly wide variety of phenomena.

The book begins with space and time. It estimates the lifetimes of Earth and of the Universe, and the sizes and distances in the visible Universe. Two major forces are obvious to us from daily life, gravitation and electromagnetism. Their characteristics are outlined and related to the variety of phenomena to which they give rise. We

explore the Solar System, understand the Milky Way as our galaxy, and relate it with far-off galaxies, made visible by telescopes. On Earth, we explore the structure of the atom and learn of its tiny nucleus, first deduced from Rutherford's alpha-particle scattering experiments. Both electrons and light show both particle and wave characteristics, and we soon reach quantum mechanics, with discrete energy levels for atoms, each level corresponding to a standing wave of electrons circulating about its atomic nucleus. The Coulomb force accounts for the binding of these circulating electrons to the nucleus, and also for the forces between atoms. We are shown the most common bonding mechanisms between atoms, and use the atoms as building blocks to make molecules, crystals and other solids and liquids, and so account for the materials around us as due to electromagnetic forces.

Next we are led to still shorter distances, to the nuclear and subnuclear worlds. The atomic nucleus consists of protons and neutrons, bound together by strong nuclear forces, and we soon understand why the stable nuclei are limited in number. An atom's chemistry then depends primarily on the proton number of its nucleus, and a pure chemical element can have various atomic mass values, corresponding to the number of neutrons in its nucleus. The ancient dream of transmuting other elements into gold is now seen to be possible, although so expensive that it has no financial value.

Recent experiments on the scattering of electrons and neutrinos by nuclei have shown that the nucleons themselves are

composite, each containing three quarks. The rich spectroscopies of atoms and nuclei, each due to their multiplicity of component particles, are now paralleled by a spectroscopy of nucleons, due to excitation of the motions and spins of their component quarks. These experiments also show us that quarks, as well as electrons and neutrinos, have no detectable structure larger than 10^{-16} cm.

We turn now to our terrestrial environment. Earth's long history is traced out, great changes occurring in its surface structure long before Man's emergence in recognizable form through evolutionary development. Thus, we come to the story of Life itself. The great steps are traced out, although not in detail, for we do not know them. We understand how the basic molecules needed to make up living material could form, perhaps through lightning flashes on the Earth's surface. The energy falling on Earth from the Sun was essential for the subsequent development of more complex organisms. Earth became covered in green, due to the chlorophyll which uses the energy reaching us from the Sun so efficiently. In the course of time, more complex organisms evolved, their structure being determined by the double-stranded DNA molecule, a thread carrying instructions for the construction of a living being, a thread replicated in its every cell. This evolution from simple to complex organisms, to living beings and to *Homo sapiens*, cannot be traced in detail but the general principles are outlined. The important role of mutations in this development is emphasized, and we see how the life on earth today could plausibly have

resulted from such an evolutionary history. Finally, we recognize that evolution in Man's behaviour has become rapid relative to his lifetime, since mankind now gains new knowledge quickly and disseminates it efficiently, so that new situations for humanity can now develop in the space of years. This evolution is of a new kind, not concerned with Man's structure but with his ability to control and change his environment. It is still quite unclear where this new evolution may lead us.

This story is recounted in simple terms, with compelling logic. The original lectures were for a high-school audience, and this extended version is still suitable for them. But it has much interest for all, and I hope that it will become more widely known. It could perhaps be simplified more, or extended in several places where questions are left unanswered. The book will be valuable for scientists, since few are familiar with all of the areas covered. The presentation is stronger and more tightly argued in the earlier chapters concerned with chemistry and physics than in the chapters concerning Life, where the author's knowledge comes from other scientists. The story of Life is much more complicated than that of inorganic matter, and can only be outlined in such a short space.

Each reader will have his own short-list of significant topics not covered in this book. For example, the uniqueness of our situation on Earth might have been underlined by a brief comparison of Venus with Earth; why have two planets of such similar constitution, mass, and so on, turned out to be so different today. Again, neutron stars are well known now, some of them as pulsars, of which there are many in the sky, but they are not mentioned; nor are quasars nor black holes, but these are not yet understood and they lie on the edge of Einstein's gravitational theory, which the author consciously chose to omit. At the other extreme, the book gives no hint of the complexity of the quark world; we know 15 kinds of quark today, and other fundamental entities are also needed. There is no mention of the weak-decay interactions, the unification of which with electromagnetism to form 'the electroweak interaction' has been such a major theoretical triumph recently. Such topics would perhaps complicate the story without adding much enlightenment, but still they are part of our everyday world. Even the strange particles are being formed around us by the cosmic radiation incident on Earth, although they quickly decay, and this cosmic radiation can be made visible with simple equipment which can be constructed in any home; it is part of our environment. Perhaps the book should have a few appendices, which could take the interested reader further towards the extreme boundaries of present research. I found few errors in the book, but it is stated on p.179 that the highest energy accelerator today, producing proton beams up to 500

GeV, is in operation at the Brookhaven National Laboratory, Long Island; it is actually at the Fermi Laboratory, near Chicago. It also puzzled me that more than half a page should be devoted to the outmoded 'continuous creation' theory of the expanding Universe, definitely excluded by observation for some time past.

In conclusion, I can only say that this book is superb and far ahead of any other global accounting of our Universe, in both the large and the small. We all have friends and relatives who question us often about

modern science; this is the book to place in their hands. It shows the physicist at his best, accounting for what he sees by simple order-of-magnitude estimates. The story of Life is less amenable to this approach, and so those chapters are more descriptive. However, as it now is, the book already satisfies a great contemporary need. □

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Star maps, old and new

David W. Hughes

The Sky Explored: Celestial Cartography, 1500-1800. By D. J. Warner. Pp.293. (Alan R. Liss: New York; Theatrum Orbis Terrarum: Amsterdam, 1979.) \$70. *True Visual Magnitude Photographic Star Atlas.* By C. Papadopoulos. (Pergamon: Oxford and New York.) Three volumes. Vol.1. *Southern Stars*, 1979; £100, \$225. Vol.2. *Equatorial Stars*, 1979; £145, \$325. Vol.3 *Northern Stars* (by C. Papadopoulos and C. Scovill) publication due June 1980.

SINCE the dawn of history, mankind has been a fascinated observer of the heavens. The stars were arranged into groups of constellations and names were given to these constellations and also to many of the brighter stars. However, without a good celestial map the night sky simply dissolved into a trackless confusion of points of light. Maps were also a simple means of recording the knowledge gleaned from celestial observations. In the old days the constellations were depicted by artistic, symbolic figures, and many of the early star maps and globes became much more than just scientific aids but were also beautiful works of art in their own right.

The first important extant star catalogue was compiled by Ptolemy in AD 150 and contained 1025 stars grouped into 48 constellations. Ptolemy's work was the cornerstone of mediaeval star catalogues and, for more than 1,400 years, Islamic and European astronomers limited their observations to the stars listed by Ptolemy. The catalogue of Al-Sufi in the tenth century, Ulugh Beg's Alfonsine Tables in the fifteenth century and the Copernicus catalogue in the early sixteenth century were simply revisions of Ptolemy. These catalogues were usually just lists of stellar latitudes, longitudes and magnitudes.

Most astronomers found that a graphic form was more useful than a catalogue, and so maps of the sky were introduced. The first flat star map of any significance was published by Albrecht Dürer, the great Nuremberg artist and mathematician, in

1515. The date reflects both the development of printing and the upsurge in the study of astronomy initiated by such worthies as Copernicus, Regiomontanus and Piccolomini. The era of great celestial cartography probably ends with the publication of John Elert Bode's *Uranographia* in 1801. Bode used 99 constellations and included 17,240 stars down to eighth magnitude. After Bode the constellation boundaries became more rational, the constellation figures slowly disappeared and star maps lost much of their charm and beauty becoming the dull procession of differing sized dots we know today.

Deborah J. Warner, the curator of the History of Astronomy at the National Museum of History and Technology, Smithsonian Institution, has produced a comprehensive catalogue of all the flat star maps printed in Europe between the fifteenth and early nineteenth centuries. Also included are details of a few globes for comparison. It is a work of considerable scholarship and, as a source of detailed information, will be a much valued reference book for decades to come. The listing is alphabetical and the work of each cartographer is analysed according to the number and size of maps produced, the limiting star magnitude, the co-ordinate system adopted, the date and place of publication, and the non-Ptolemaic constellations introduced. It is intriguing to note how certain constellations come and go. For example Lalande charted Felis, his own cat, and also introduced his telescope as Quadrans Muralis. Thomas Young squeezed the Battery of Volta into the space between the head and hooves of Pegasus. Julius Schiller even attempted to de-paganize the complete zodiac by introducing the twelve apostles in the place of Pisces, Aries, Taurus, Gemini and so on. The Warner catalogue is profusely illustrated in black and white and the beauty of these illustrations is most impressive. Obviously only a few of the maps were coloured and even this would probably have been a laborious task of hand water-colouring. It is hard to fault this book but may I make a suggestion for a new venture. If Deborah Warner produced a large-format book, with some colour prints depicting the best artistic

representations of each constellation, I'd be first in the queue with my money.

The transition between star maps, the beautiful, and star maps, the functional tool of modern science, is illustrated perfectly by the second book under review. Papadopoulos has produced a large set of maps by photographing the sky through a four-element astrographic objective lens manufactured by Carl Zeiss (Oberkochen). This lens had a diameter of 12.5 cm, a focal length of 62.5 cm and gave a negative with a usable field of 15°. Each map has been produced from the original negative by line reproduction, a system which prints only black, and has no halftones. This is ideal for stars but gives a very poor reproduction of the appearance of galaxies and nebulae.

The *Southern Stars* volume contains 120 maps covering the sky region from declination -30° to the South Celestial Pole. Each map covers an area of 11° by 11° and there is a minimum overlap of at least 1° between neighbours. The *Equatorial Stars* volume contains 216 maps and covers the region between -30° and +30° in declination. A set of transparent co-ordinate overlays are provided, six for Vol. 1 and three for Vol. 2. The system is converted to 1950.0. Each map measures about 33 cm by 33 cm. Volume 3, *Northern Stars*, will be published later this year.

The atlas is designed to show all stars which are brighter than visual magnitude 13.5 and, to quote the author, "the final print produces star images with easily readable differences in their diameters according to their magnitude". The accuracy with which the magnitude can be assessed obviously depends on the accuracy with which the star image diameters have been produced on the map and on the accuracy of measuring these diameters (a star of third magnitude is about 1 mm across). Superficially each map appears like a white sheet speckled with tiny black dots, surrounded by a black border with right ascension and declination markings on them so that the transparent grid can be positioned. To find your way between maps you are given a rather inadequate, small-scale, chart index. You actually need at least a copy of Antonin Bečvář's *Atlas Coeli* and preferably a set of Smithsonian Astrophysical Observatory star charts as well. The main advantage of the Papadopoulos atlas is that it goes down to visual magnitudes 13.5 as opposed to the SAO which stops about ninth. The main disadvantage is the price, but assuming you need to go down to 13.5 mag (and I would be very surprised if the number of astronomers who do really need to do this is more than miniscule) £245 for a collection of 336 Papadopoulos maps — Vols. 1 and 2 — is what you have to pay here. I must confess that I won't, and neither will my university. □

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Thriving young discipline

Martin Daly

Human Sociobiology: A Holistic Approach. By D.G. Freedman. Pp.192. (Free Press/Collier Macmillan: New York and London, 1979.) £8.50. *The Genesis Factor.* By R.A. Wallace. Pp.256. (William Morrow: New York, 1979.) \$9.95.

HUMAN sociobiology seems to be a thriving young discipline, if a spate of books and new journals over the past two years is to be taken as evidence. Perhaps much of this activity will eventually be seen to be the cloaking of old ideas in new jargon, but much of it is truly synthetic. Sociobiology is gaining adherents among social scientists because, rather than contesting their established theoretical approaches, it is for the most part metatheoretical to their usual levels of analysis. Theories of sex role socialization, for example, may accurately describe processes by which masculine and feminine behaviour develop, while offering no insight into the cross-cultural consistency of the outcomes. Or a potlatch may be explained as a status competition, while taking it as a given that status is a commodity for which men will find it reasonable to compete. Sociobiological theory attempts to deal with some of the questions that structural, descriptive theories of human action fail to address.

If a "new synthesis" can indeed be said to be emerging, it is not surprising that anthropology, the social science most informed by evolutionary thought, is leading the way. However, sociobiological schools of thought have also surfaced in economics, sociology and even political science. The laggards whose participation is most seriously missed are psychologists.

The grounds for thinking psychology a crucial missing element in a productive synthesis are as follows. Anthropologists and other scientists testing sociobiological hypotheses have been concerned to demonstrate measurable fitness consequences of extant variation in behaviour, and they have achieved some notable successes (see, for example, chapters in *Evolutionary Biology and Human Social Behavior: An Anthropological Perspective*, edited by N.A. Chagnon and W. Irons; Duxbury Press, 1979). However, a good deal of modern human behaviour is manifestly not fitness-maximizing. Must sociobiology therefore be mute about its origins? Not if appropriate models of the structure of human motives and cognitive processes can be developed. The psychological mechanisms proposed must be anchored simultaneously in sociobiological accounts of their phylogeny and adaptive significance and in predictions of their behavioural outputs, including predictive

statements about the kinds of circumstances in which such evolved mechanisms can be expected *not* to produce adaptive outcomes. This task offers experimental psychologists a gold mine of opportunity for research and for theory development.

Daniel Freedman's *Human Sociobiology: A Holistic Approach* constitutes an early psychological foray into this rich field, and while the effort is deeply flawed, it nevertheless unearths several nuggets. The book's greatest shortcoming resides in the fact that Freedman ventures out of his depth in population biology. This produces two extremely distracting muddles. One, which surfaces repeatedly, concerns the significance of populational levels of heterozygosity. Freedman imagines that inbreeding is selected against not because of any deleterious effects of homozygosity upon the offspring of consanguineous unions, but because it constitutes "bad evolutionary planning" by reducing populational reservoirs of heterozygosity. He proceeds from this basic misunderstanding to characterize human populations as more or less heterozygous according to their marriage practices, a train of speculation which in Chapter 7 leads to several odd conclusions which leave the author needlessly vulnerable to accusations of racism.

The second major muddle is conceptually related to the first. Freedman has borrowed his University of Chicago colleague Michael Wade's suspicion of

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The First Two-Thirds of Mammalian History

Edited by Jason A. Lillegraven

Mammals have been an important part of the earth's land fauna for at least 200 million years. This book offers a comprehensive review of what is known about mammalian life during the first two-thirds of its history — the Mesozoic era — before the extinction of the great reptiles. The book provides biologists and geologists with encyclopedic listings of fossil locations, recognized taxa, and temporal-geographical occurrences of genera along with descriptions of the anatomical features of each group and extensive bibliographies. Interwoven with the summaries of data are valuable interpretive — sometimes admittedly speculative — discussions of the origins of mammals, their patterns of distribution, and their biological, behavioral, and evolutionary relationships during the "dark ages" of mammalian history. Illus. 320 pages £21.00, cloth £5.75 paper.

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"selfish gene" reductionism without apparently grasping the rationale for that suspicion. The undisputed fact that social groups have complex interactive properties which may be mistaken by researchers focussing attention on individuals is thoroughly confused with the question of the roles of group versus individual selection in the evolution of adaptation. I am not cavilling on this subject, for the author's imagined critique of individual selection is a major, recurring theme of the book. He begins Chapter 4 with this paragraph (page 45):

I hope I have by now established that both the selfish-gene approach from below and the group selection approach from above are needed for an adequate explanation of social behavior, no matter the species. When we get to primates, the need for a dual causality model rather than a one-way model is emphasized since primates can act with clear selfishness or in concert, as the situation demands.

By this point, sociobiologically sophisticated readers may have decided to take a walk, and that is a pity, for the book's strengths lie just ahead.

One strong feature is Freedman's cross-cultural review of sex differences in the behaviour of human children. Cultural anthropologists have seldom standardized methodology or collected quantitative data, so the psychologist's touch is valuable here. Among the many interesting phenomena described are the prevalence of acknowledged hierarchies of "toughness" among small boys and the irrelevance of the same dimension for girls. Also intriguing are data on children's drawings, in which thematic sex differences are highly consistent across cultures even as substantial between-cultures variation is manifest.

Fifty student research projects carried out under Freedman's aegis (and outlined in a fifty-page appendix) are discussed in the text. Though highly variable in quality, these studies introduce a number of innovative methods and provocative findings. For example, modified TAT cards (pictures about which subjects are invited to tell a story) are used to assess the emotional impact and status significance of facial hair and of relative height, with results that sometimes contradict the respondents' professed attitudes. In a similar vein, the dimension of kinship is added to another standardized psychological instrument, Kohlberg's Moral Dilemmas, in order to provide evidence that people apply different moral codes to kin and non-kin. The use of such techniques should inspire readers interested in developing a socio-biological psychology.

The concluding chapter, entitled "Biology or culture?" begins with a not altogether successful attempt to dismiss such unproductive either-or-ism: "There is no way to separate the genetic and the learned for they are permanently glued together, and that is true everywhere in nature, including man". Heaven knows what is meant by "learning" if it is

manifest "everywhere in nature", but most readers will probably find the sentiment acceptable if not the logic offered in support of it. Freedman then reviews his own convincing evidence for population differences in human infantile behaviour and temperament, proceeds to speculate plausibly that such differences may be causal to non-arbitrary cultural differences, and somehow concludes that racial "antagonisms are more or less built into the species" and that "the only sound solution lies in miscegenation". One is left perplexedly leaving back, vainly trying to reconstruct the extraordinary logic of this argument.

Robert Wallace's *The Genesis Factor* is blurred as "the first popularly written book on sociobiology". If popular writing consists in talking down to one's readers and in disdaining to cite sources, the characterization is correct. Popular writing also apparently entails a suspension of respect for fact. Here are just three examples: parental care occurs in "only a few isolated species" of fishes (page 37); gorillas are "highly promiscuous" (page 76); and the administration of androgens to infant female mice transforms them into killers

without interfering with normal oestrus (page 116). Even where a particular experiment is under discussion, Wallace is liable to serious mis-statement of the results, as in his treatment of Harry Power's mountain bluebird removal experiments (page 179). Running roughshod over such disparate subjects as IQ and the alleged adaptive value of death, Wallace's book will regrettably reinforce antibiological prejudices of some social scientists while proving an embarrassment to thoughtful biologists. It ends on an even more remarkable note than Freedman's book, namely the author's personal foreign policy recommendations for the United States government! With friends like Wallace, sociobiology has no need of enemies.

Let us hope that we will see more and better empirical work of the sort initiated by Freedman. And may we have fewer grand statements of the policy implications of human sociobiology, at least until the discipline grows up a little. □

Martin Daly is an Associate Professor of Psychology at McMaster University, Hamilton, Ontario.

Orcadian heritage

Euan W. MacKie

Investigations in Orkney. By Colin Renfrew. Pp.234. (Society of Antiquaries: London. Distributed by Thames and Hudson: London, 1979.) £20.

UNTIL the recent oil discoveries the Orkney Islands seemed remote from and peripheral to modern Britain, yet several times in the past they have been at the crossroads of important movements of people and influences. These usually came either up the western seaways from Atlantic Europe, like the stone tomb-builders of Neolithic times and elements among the inventors of the Iron Age tower-forts known as brochs, or, like the Norsemen and the Early Bronze Age Beaker people respectively, westwards from Scandinavia or north-westwards from the Low Countries or the eastern mainland of Britain. Like the churning cross-currents of the Pentland Firth, these genetic and cultural mixtures often produced periods of striking local developments in the islands. The sandstone of which these are formed provided ideal building material and some remarkable developments in drystone architecture resulted.

Archaeological research in Orkney has been sporadic in the past with long periods of inactivity, but the early 1970s saw a burst of new work by visiting excavators. Sites

like the Neolithic dwellings at Skara Brae and Knap of Howar, the Pictish and Norse village at Buckquoy, the prehistoric and early historic settlement at Skaill and the Neolithic chambered tomb at Quanterness (the main subject of the book) were explored with modern techniques for the first time. Knowledge is still advancing rapidly through the efforts of a new resident team of archaeologists financed by the Scottish Development Department, the North of Scotland Archaeological Services Unit based at Finstown.

Professor Renfrew has long been concerned to explain in modern terms the dramatic changes visible in the prehistoric archaeological record, such as the appearance of the chambered burial mounds of Atlantic Europe at around 4200 BC, and he favours hypotheses of indigenous development rather than any involving the diffusion in some way of ideas and skills from more advanced cultures. The radiocarbon dates for the earliest European chambered mounds have in any case shown them to be older than those of the Early Bronze Age in the Mediterranean world which were originally suggested as prototypes. Another aspect of this new approach is the interpretation of the tombs in terms of the kind of societies which built them and of the environment in which these people existed. The cairn at Quanterness is the first in Orkney to have been investigated with these general problems in mind.

One example of the detailed mass of evidence collected may be cited.

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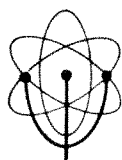
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The Knowe of Yarso, Rousay.

Quanterness is an architecturally sophisticated tomb and its period of use was determined by radiocarbon measurements to between about 3200 and 2500 BC. (This itself is important as it shows clearly that the tomb is relatively late in the Neolithic period and in the Orkney series, refuting again the old diffusionist theories which demanded that the most

sophisticated buildings were built first, by the original skilled colonists.) The number of individuals buried collectively — generation after generation over a period of 550 ± 180 years — in this great roofed stone vault was estimated from the thousands of disarticulated human bones recovered to be about 400. Both sexes and most age groups were represented, and the

conclusion drawn is that all the members of a small farming community of 13–20 people were buried in the cairn.

These results, and those of the many other detailed researches carried out, form a landmark in our understanding of Orcadian Neolithic society — a mass of hard evidence which will provide a minimum standard for all future work. My only doubts concern some of the conclusions drawn from the data, particularly the failure to consider in any way — even simply to dismiss it — the alternative social interpretation of these cairns. This, argued in some detail in my book *The Megalith Builders* (reviewed in *Nature* 270, 644; 1977), suggests that the people buried in the cairns could have been the members of a non-farming section of the Neolithic population, most probably of religious orders which were linked genetically and by shared architectural and funerary traditions to the other cairn builders all over Atlantic Europe. The work at Quanterness has not disproved this interpretation unless this is considered to have been achieved by the author firmly looking the other way. □

Euan W. MacKie is Assistant Keeper of Archaeology and Anthropology in the Hunterian Museum, University of Glasgow, UK.

Immortalized in a virus

D.H. Watson

The Epstein-Barr Virus. Edited by M.A. Epstein and B.G. Achong. Pp.459. (Springer: Berlin, Heidelberg and New York, 1979.) £34.50.

EPSTEIN-BARR (EB) virus was discovered in 1964 by the two workers immortalized in its name; they identified particles with the characteristic morphology of the herpesvirus family in lymphoblasts cultured from a lymphoma of a type first described in African children by Burkitt in 1958. The first chapter in this volume is an account by Epstein and Achong of this early work in which Epstein's inspiration was to examine cells cultured from the tumour after an earlier fruitless search in biopsy cells.

Since 1964 a formidable body of published work has been amassed by workers from a wide variety of disciplines using an impressive array of biochemical and biological techniques. The remaining articles in this volume, written by a number of those who have made notable contributions, summarize and review progress since 1964.

Serological studies played an important initial role, and an early chapter by Ernberg

and Klein is appropriately devoted to an account of EB antigens. This sets the stage for a description by the Henles of their notable serological studies, which not only identified EB virus as distinct from other herpesviruses but most unexpectedly showed that the virus was ubiquitous, often occurring in leukocytes of healthy donors in many parts of the world. This led to the link between the virus and infectious mononucleosis and also with a second rare malignancy, nasopharyngeal carcinoma. Plainly the major stumbling block was the lack of a unique or even consistent relationship of the virus to the malignant conditions.

Molecular hybridization studies, excellently described in articles by Pagano and Shaw and by zur Hausen showed, however, that virtually all nasopharyngeal carcinomas and African (if not American) Burkitt lymphomas contained virus DNA.

There still remained the difficulty of explaining why such a widely distributed virus apparently produced tumours in restricted geographical areas. Burkitt himself suggested that a malarial co-factor might account for the lymphoma but although, as indicated in a later chapter by Epstein, there is supporting evidence for this idea, it would seem that it cannot be a unique co-factor given the comparative rarity of the lymphoma, even in its favoured locale, in doubly infected individuals. Others have argued for strain

variations in the virus and this is discussed in the chapter by Pagano and Shaw as well as by Miller.

A further twist to the tale is provided by the recognition that the virus DNA may sometimes exist in the cell in a non-integrated ('episomal') form and at other times be integrated as described here by Adams. He, like Pagano and Shaw, envisages the rare change to malignant transformation from non-malignant proliferation as being mediated by insertion and operation of transforming sequences.

The other chapters are equally absorbing, dealing with aspects as varied as the recent IARC prospective sero-epidemiological survey (de Thé; see also *Nature* 274, 756; 1978), prospects for vaccine control (Epstein), and comparative studies on oncogenic herpesviruses (Deinhardt) — to make what must in the space available be an arbitrary selection.

The volume will be useful to those from many disciplines in which EB virus has become important, in providing them with a useful statement of the current situation. Even those directly involved in the large numbers of publications on the virus will welcome the presentation in one place of such a wide-ranging survey. □

D.H. Watson is Professor of General Microbiology and Head of the Department of Microbiology, University of Leeds, UK.

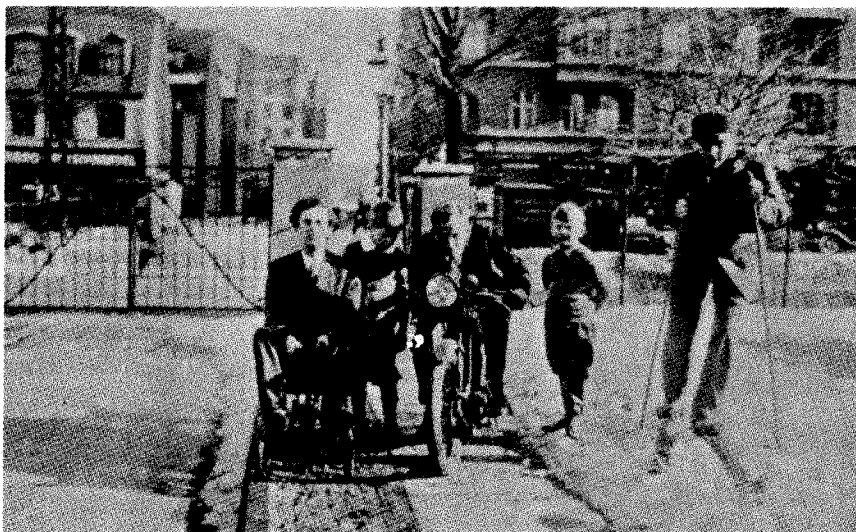
They touched the nerve of the Universe

V.F. Weisskopf

The Early Years: The Niels Bohr Institute 1921-1930. By Peter Robertson. (Akademisk Forlag, Denmark, 1979.) £6.

THIS book is a short account of the first ten years of Niels Bohr's famous Institute for Theoretical Physics. This institute was the scene of one of the most revolutionary and far-reaching developments in natural science, the birth of quantum mechanics. In some ways it was also the first international scientific institution, not in the sense of being internationally managed, but by having assembled, for shorter or longer periods, groups of young and productive physicists from many countries. Their names include most of the great leaders in physics such as F. Bloch, H. Casimir, P.A.M. Dirac, P. Ehrenfest, G. Gamow, W. Heisenberg, L. Landau, W. Pauli and many others. It was at that time, and with those people, that the foundations of the quantum concepts were created. In lively discussions the deepest problems of the structure of matter were

At the Institute early in 1931, Lev Landau, George Gamow and Edward Teller with two of Bohr's sons, Aage and Ernest.



conventional bonds, and a spirit of joy that can hardly be described by anyone who was not there at that time. In this great period of physics Bohr and his collaborators touched the nerve of the Universe. They were able to penetrate into the inner workings of nature that had been a secret up to this point. In the course of a few years only, the basis was laid for a science of atomic phenomena that grew into the vast body of knowledge known to us today.

The book by Robertson tries to recount the events that led to the foundation of that institution and the happenings during its first decisive and creative decade. It is the result of a painstaking research into the history of this unique institution. The author gives a lively and easily readable account in a little more than 150 pages, but one notices that he was not there at the time. We read about the early attempts to create a centre of international physics after the ravages of the First World War had interrupted scientific collaboration;

we read about the bureaucratic and financial difficulties that were overcome only with support by the Rockefeller Foundation; we read about the different scientists who came to the institute and about their work and their conversations with Bohr. One of the most attractive features are the many photographs of the institute, of the physicists, singly and in discussions, and of conferences and meetings that had taken place in Copenhagen and elsewhere during that period.

Reading this book and looking at the pictures is a nostalgic experience for those who had the privilege of being in contact with Bohr's institute in one way or another. I am not sure how much of the spirit and of the intellectual achievements of the institute is transmitted to a reader who has not had that great privilege. □

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George Gamow and Wolfgang Pauli on a lake steamer in Switzerland.

brought to light. N. Bohr was the acknowledged leader; he created a style of thinking, the "Kopenhageuer Geist". The greatest among his colleagues, he was acting, talking and living as an equal among a group of young, optimistic, jocular, enthusiastic people, approaching the deepest riddles of nature with a spirit of attack, a spirit of freedom from

Ape language, cognition and culture

Carolyn A. Ristau

The Ape's Reflexion. By Adrian Desmond. Pp. 288. (Blond and Briggs: London; Dial Press/James Wade (Dell): New York, 1979.) £7.95, \$10.95.

THE APE'S REFLEXION is man; the possibility of the ape reflecting has created recent controversy. Is the ape capable of some primitive language; how extensive are his cognitive abilities; does the ape murder; does he have a sense of self and of death? Adrian Desmond deals with these momentous concerns in what I presume is intended to be a semi-popular book. His writing style is often fluid and he frequently achieves a sense of immediacy, seeming to

give an eye-witness account of the research. One can easily nestle down with his book for a few evenings' light reading. The sense of an eye-witness account is apparently not based on any visits by the author to research in progress. This is unfortunate, for an objective, careful and critical reporting of anecdotal information would be a useful addition to the literature on the ape language projects. The scientific literature suffers from a lack of such information while the popular literature abounds with dramatic and careless reports.

Briefly, Desmond's book concerns itself primarily with the ape language research projects, the neuroanatomical comparison of man and chimpanzee, tool use and proto-cultural activities of chimpanzee and early man, and some of Jane Goodall's investigations at the Gombe River Stream Preserve. There are numerous direct quotations from researchers' studies, an index and footnotes. The footnotes,

located at the back of the book, indicate references and sometimes offer detailed discussions. An alphabetical list of references and more such discussion would have been useful additions. The book also contains 24 pages of black and white photographs, predominantly of chimpanzees from the language projects and the Gombe River Stream.

Desmond considers man's close genetic relationship to the chimpanzee, citing 99% similarity in DNA nucleotides. Yet he does not note that the 1% difference appears to control timing and sequence of genetic expression, a mechanism by which organisms can develop very differently from each other. Furthermore, overall genetic similarity or dissimilarity need bear no necessary relation to the similarity of particular attributes of any two species. A dolphin is physically very different from man, yet may have certain cognitive abilities that are similar to man's. Desmond's comparisons between the Gombe River chimpanzee killing and man's act of murder, as well as many anecdotes from the ape language projects, are other examples of dramatic interpretations of data that need further analysis.

Desmond does raise important points. For example, in Desmond's consideration of the ape language work, Wittgenstein's aphorism, "If a lion could speak we could not understand him" (p.33), is quoted in reference to the naive assumption often made by trainers that chimpanzees use signs like 'please' and 'think' with the meanings assigned to them by humans (p.35).

Desmond also describes the recent investigation of Savage-Rumbaugh *et al.* (p.107) into the meaning of an utterance. Their chimpanzees seem to have the ability to use a lexigram or geometric design such as the design for 'key', in a highly specific context, but become bewildered upon its application to a wider array of contexts.

That is, the chimpanzees seem to recognize that use of the lexigram for key will initiate a chain of events that eventually leads to their obtaining food with a key, but do not seem to understand that the lexigram is the 'name of' a key. Gradually some kind of understanding closer to that level does seem to be achieved, but the research makes it clear that a chimpanzee's (or young child's?) appropriate use of a 'word' in a limited number of contexts does not imply understanding of the word.

However, as Desmond notes, words *can* 'map on' to thought. Premack conceptualizes his 'ape language' investigation as permitting apes to use words to map out conceptual structures the animal already possesses. The end of the work is thus not to teach an ape language or to wonder how linguistic the apes' abilities are or are not, but to use the ape 'language' as a tool for revealing some of the apes' cognitive capacities.

Throughout, Desmond wisely emphasizes that we cannot measure apes with a human yardstick.

Another of the more positive parts of Desmond's book is his exciting account of the trail of discoveries initiated by analysing soil samples and leading, finally, to the discovery, in the molecules of soil, of flowers at the grave of a Neanderthal man. Here was an indication of some ceremony for the dead, a remnant of culture in so early a hominid.

In all, Desmond does consider fascinating issues and offers some insightful thoughts. But one wishes at least for less wordiness, more dispassionate writing and less uncertainty about the validity of his interpretations. And does the reader need to find phrases like "Oh Darwin, where art thou now?" □

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Coulson's Valence

Linus Pauling

Coulson's Valence. Third edition. By R. McWeeny. Pp.434. (Oxford University Press: Oxford, 1979.) Hardback £17.50; paperback £8.50.

A SIMPLE but very powerful theory of chemical valence and the structure of molecules, especially of compounds of carbon, was developed in the period between 1852 and 1874 by E. Frankland, F. A. Kekulé, A. S. Couper, A. M. Couper, A. M. Butlerov, J. H. van't Hoff and J. A. Le Bel. It was extended in 1893 by A. Werner to include complexes of metals, which he identified correctly from chemical evidence as representing tetrahedral, square planar or octahedral coordination of ligands about a central metal atom. An important contribution to

the theory was made in 1916 by Gilbert Newton Lewis, who described the chemical bond as a pair of electrons held jointly by two atoms and who emphasized the stability of molecular structures in which each atom is surrounded by the same number of electrons as in a neutral noble-gas atom. Important contributions, such as the principle of electroneutrality and possible covalence as large as eight (for nickel in nickel tetracarbonyl), were made by Irving Langmuir from 1919 to 1921.

Despite some clarification introduced by the new ideas about the electronic structure of atoms and molecules, the theory of valence and the structure of molecules and crystals clearly remained incomplete. Many questions were asked in the first half of the 1920s that could not be answered; for example, why are the aromatic hydrocarbons more resistant to hydrogenation than other unsaturated hydrocarbons, why do complexes of palladium(II) and

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platinum(II) have the square planar structure whereas those of zinc(II) and cadmium(II) are tetrahedral, why are most compounds formed from the elements with a decrease in enthalpy, and most basic of all, why does sharing a pair of electrons between two atoms lead to a bond that holds the atoms together? The answers to these and many other questions were found in the period 1927 to 1933 through the application of the newly discovered theory of quantum mechanics to the problems. In 1927 ϕ . Burrau published his quantum-mechanical treatment of the one-electron bond in the H_2^+ molecule-ion describing the first molecular orbital, and in the same year E. U. Condon treated the electron-pair bond in H_2 by the molecular-orbital method, and W. Heitler and F. London treated the same problem by the prototype of the valence-bond method. In 1926, and in greater detail in 1931, I introduced the concept of hybridization of bond orbitals, and between 1931 and 1933 I discussed the quantum-mechanical basis of tetrahedral, square planar and octahedral coordination, ionic-covalent resonance energy, the electronegativity scale, magnetic properties of complexes, the structure and properties of aromatic and conjugated molecules, and several other matters relating to valence and molecular structure.

Between 1928 and 1931 the quantum-mechanical theory of the resonance of a

system between two or more possible structures and the consequent stabilization of the system by resonance energy had been discussed by John Slater, by me and by E. Hückel, and Slater had, in 1931, formulated his expression for the wave function representing an assigned valence-bond structure for a molecule. Many detailed quantum-mechanical calculations were being made of molecular properties by both the molecular-orbital method and the valence-bond method, and in 1931 both Salter and I published closely similar short papers to point out that, whereas these two methods in their simplest forms gave somewhat different results, when refined they approached each other, ultimately becoming identical.

During the 50 years since these events took place molecular quantum mechanics has made great strides. Many extensive calculations have been carried out, mostly with emphasis on molecular orbitals. I chose, however, to attempt to refine the theory of the chemical bond in a semi-empirical way, incorporating the ideas and principles of quantum mechanics. The results of this effort, in which I adhered as closely as possible to the ideas of classical valence-bond theory, were described in 1939 in my book *The Nature of the Chemical Bond* (third edition Cornell University Press: Ithaca, New York, 1960).

In the first edition of his book *Valence* (Oxford University Press: Oxford, 1952),

Professor Charles Coulson emphasized the molecular-orbital description of electrons in molecules, with rather little discussion of empirical information. This policy has been followed by Professor McWeeny in preparing the much enlarged third edition. The result is that the book contains only a small amount of information about valence. There is no significant discussion of oxidation number, the range of values of the valence shown by many elements, and a number of other aspects of valence theory. Instead, as the author states in his preface, the book provides an introduction to quantum mechanics and a discussion of the application of quantum mechanics to the structure of molecules, with emphasis on the molecular-orbital method (which the author describes as having largely superseded the valence-bond method). The level of the discussion is for the most part rather high, but there are some surprising exceptions, such as the failure to derive the wave functions for the hydrogen atom. The book provides a survey of molecular quantum mechanics and of some aspects of structural chemistry. An isolated student without previous knowledge of quantum mechanics might find it difficult, but it could serve well as the textbook in a course, the purpose for which it was written. □

Linus Pauling is Research Professor at the Linus Pauling Institute of Science and Medicine, and is the holder of two Nobel Prizes.

Soviet innovation

Christopher Freeman

Science and Industrialisation in the USSR: Industrial Research and Development, 1917-1940. By Robert Lewis. Pp.211. (Macmillan: London, UK, 1979.) £12.

OVER the past 20 years the Birmingham Centre for Russian and East European Studies has established a high reputation for carefully documented research on Soviet R&D and industrial innovation. This book will certainly enhance that reputation and in many ways is even more interesting than its predecessors. Although it is entirely concerned with the inter-War years, the great interest of this period is that it was the formative time when the structure and procedures of the Soviet industrial innovation system became firmly established. It is also remarkable how many of the post-War debates in Western Europe were anticipated in the pre-War controversies in the USSR. Who remembered, for example, that the Rothschild "customer-contractor" principle was enthusiastically introduced in the Soviet institutes 50 years before its application here?

The new Soviet state inherited a science-technology system in 1917 which had many of the characteristics of today's under-developed countries — dependence on foreign technology and an almost complete lack of industry-based R&D. The Soviet leaders attempted from the outset to build up their own industrial R&D system, and the expansion in the 1920s and early 1930s was much more rapid than elsewhere. Indeed the Soviet Union has probably spent a higher proportion of its national income on scientific and technical activities than any other country for about half a century now. In absolute terms, this was, of course, still a fairly small total in the 1930s and as Robert Lewis is careful to point out "R&D" is only one part of the total rubric "science and technology". Definitions of R&D differ between Eastern and Western Europe and in any case were only formulated after the Second World War.

The first few chapters of the book document the growth of the Soviet science-technology system during the inter-War years with scrupulous attention to detail. (In a 200-page book, 50 pages are devoted to sources and methods.) The next few chapters review the organization of the system and the various attempts to control, coordinate and plan it. Finally, the last three chapters analyse this experience

critically with a particularly interesting discussion in Chapter 10 on "Yesterday and Today". They seek to answer the question: why has the return on the massive Soviet R&D investment been so unsatisfactory?

Robert Lewis concludes that the major structural weakness of the Soviet industrial R&D system was the failure to build up a strong R&D capability at the enterprise level and that this weakness persists to this day despite a succession of attempts to overcome it. Shortages of skilled manpower and equipment as well as the desire to avoid expensive duplication dictated the early decisions to concentrate the industry-related R&D in central institutes for each branch of industry. Ever since then, there has been a continuous theme of complaint that the central institutes were too remote from the real problems of the factories and conversely that industry had neither the capacity nor the incentive to apply the results of the R&D conducted in the central institutes.

This problem is by no means confined to the Soviet Union or to centrally planned economies. Even within large private enterprise, it is a familiar theme of the R&D management literature. In one form or another it is likely to persist even in the best-managed systems as it reflects a real difference in preoccupation and pers-

pective between those primarily concerned with the management and improvement of an existing production system and those concerned with potential changes in products and processes including the total displacement of existing systems. However, there seems little doubt that the problem was particularly acute in the Soviet Union as institutional barriers were reinforced by geographical and political constraints. Lewis shows that, despite many declarations of good intentions about decentralization, the R&D establishment remained overwhelmingly concentrated in the Moscow and Leningrad regions. He calculates that a decade of pressure resulted only in a reduction of the proportion employed in these two regions from 80% to 75%. Again, this is by no means exclusively a Soviet problem.

The final chapters suggest, although not very explicitly, that the most important specific Soviet weakness has been the over-centralization of decision-making associated with Stalinism. It is of some interest that already in the 1920s Pyatakov and other members of the "Left" opposition were advocating the wholesale decentralization of industrial R&D to the enterprise level. The combination of the output target system, the import of 'turn-key' foreign technology, the shortages of skilled people and the decimation of the intelligentsia in the purges of the 1930s meant that the various attempts which were made to strengthen enterprise-level R&D had little effect.

The major exception which Lewis points to was the aircraft industry, and the discussion of its peculiarities in Chapter 9 is one of the most interesting in the whole book. This industry was a high priority for the leadership throughout the 1920s and 1930s which found expression both in a relatively lavish level of funding and in a readiness to change the organizational system in order to get the results so urgently demanded by Stalin and other leaders, who apparently showed an almost daily interest. Whereas in other industries it was difficult to get adequate resources for prototype and pilot plant work, in the aircraft industry several alternative designs were often promoted simultaneously right through to the prototype test stage in development. In the aircraft industry too, the single central research and design institute gave birth to a pluralistic structure already in the early 1930s which included the rather successful flexible design groups assembled under the leadership of individual designers and sometimes located at factories.

Centralization to a high degree certainly persisted in the aircraft industry, but it was combined with a real choice in design and development based on autonomous effort and with relatively good communications with the production systems. An interesting point made by Lewis is that sometimes factory personnel were brought into the design and development teams at a fairly

early stage. He does not discuss it, but one may guess that this industry also made good use of its own products to ease the physical communication problems of the USSR.

By preventing Germany and Japan from embarking on expensive aircraft, nuclear and other military R&D projects for two decades after the Second World War, the USSR and its War-time allies conferred upon them the inestimable blessing of concentrating most of their best engineering and scientific talent on the industries which really mattered for economic growth and international trade performance. The results are now increasingly apparent. The British in a mild way since 1945 have suffered from the Russian problems of the 1930s — too high a priority for R&D in prestige defence-related industries and relative neglect of the

rest. As in the USSR, a very high proportion of our best science and engineering talent was locked into this system for 30 years, while the mechanical engineering industries, vehicles and other mundane branches of the economy did not have the capacity to design and develop new products to keep pace with world competition. To this day, they do not have and often cannot get the electronic engineers they need to re-design their products and processes. It would be ironical if the 'Iron Lady' in her anxiety to combat the Soviet 'menace' caught a more virulent strain of the Russian 'flu'. □

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Voyages through the cosmic landscape

D.D. Clayton

Cosmic Landscape: Voyages Back Along the Photon's Track. By Michael Rowan-Robinson. Pp.149. (Oxford University Press: Oxford and New York, 1980.) £4.50.

WHEN I began this book I was somewhat prejudiced against it because I thought it would sink from the burden of its seemingly unnatural standpoint. After all, astronomical knowledge has exploded from the combined measurements of astronomical objects by instruments responsive to the different spectral bands. Yet, here Michael Rowan-Robinson presents "cosmic voyages in each of the main wavelength bands of contemporary astronomy". He finds himself "taking this metaphor of voyage literally, and describing how I think things would look from a viewpoint different to that seen from earth. Certain objects in the cosmic landscape figure in several different wavelength bands and so our picture of them builds up only gradually from the succession of voyages in each band". Surely, I thought, the only way to discuss the Crab Nebula, or QSO 3C 273, would be from the simultaneous knowledge from all types of measurements, and not from a succession of half-blind glimpses. But in the end my negative predisposition was overcome by an informative and often poetic presentation. This is a very good book, although it fails by slim margins to be a great one.

The author says, "I have tried to write a book for someone who might never have read anything about astronomy". So restricted, he must at times be a teacher: "What is light?"; "What is colour?"; "How is light made?"; "What are radio waves?" are subsections of chapters in which he must also explain such phenomena as atomic and molecular structure and radioactivity without seeming to delay forever the departure of the cosmic expresses. Amazingly, he has surmounted this obstacle with as much grace as one can hope for. He does this by appealing to general matters of human interest — for example, by describing briefly the red, green and blue-light-sensitive organic pigments in the cone-shaped detector on the back of the human retina. Occasionally, he forgets this audience and uses technical terminology that can only impede the novice; for example, because he uses it he must explain that in writing O VI "the Roman numeral corresponds to one more than the number of missing electrons". The book is, I think, a bit difficult for readers without scientific backgrounds; but the author has made a good compromise between simplicity and the inspiration of real intellectual content. For this reason, I will recommend this book to nonprofessionals interested in astronomy, even knowing they will not understand some of it.

So we set off in succession on six voyages: the visible landscape; the radio landscape; the ultraviolet landscape; the X- and γ -ray landscape; the infrared landscape; and the microwave landscape. I could not discern the author's reason for this order; but neither can I criticize it as illogical or inappropriate. Perhaps putting the infrared landscape off until the fifth voyage has something to do with the author's personal research emphasis. It warms him especially to this voyage, causing him to note here, almost sadly, that totally new surveys of the sky seem to be

now a part of history, and then to the confession: "For me it has been a wonderful time to be alive and to be an astronomer. And so I try to share this experience with you".

To analyse the success of this book I must, like the author, branch out from astronomy into the larger realm of human experience. A landscape is interpreted as a partly subjective human experience, with the human at its centre. Its objective, in an artistic sense, is not the description of the separate parts of the scenery, but rather a description of the act of perceiving it. Rowan-Robinson has not idly chosen a catchy title; he has chosen it specifically to his artistic purpose. The ponderous structure of separate voyages in different wavelength bands succeeds because his purpose is not to explain our understanding of the objects in the landscapes, but rather the human act of perceiving them. He evokes, perhaps unintentionally, the experience of the child in its crib, looking out at baubles hanging above him and wondering at the meaning of a perception he cannot touch. It is a powerful undercurrent that gives

force, by its repetition, to these cosmic voyages. The reader glimpses the human race in its infancy, reaching out.

I cannot say that Rowan-Robinson intended all of this, but I believe that he did. As someone who has also attempted to enlarge the genre of scientific writing, I can only admire his effort. It falls short of greatness because the author was not sufficiently single-minded in this task. The pedagogical explanations weigh down the poetic experience. We have here the artist painting his landscape and, at the same time, explaining to us how we know that the water in the creek flows downhill rather than up. On the positive side of that coin, however, there is fascinating astronomical and physical knowledge interestingly described on each page. So it is also a book from which to learn about astronomy. For a skilful blend of these objectives, the author earns grade A. I remain fascinated with the partially fulfilled opportunities of this book as a poetic and artistic experience. I can best share this fascination by the following poem, which this book inspired me to write:

On His Reading *Cosmic Landscape*

Hanging there on strings, tiny baubles above my crib,
What would I name you if I, like they, knew words?
Jupiter, Venus, pulsar, quasi-stellar objects of my gaze,
Abstaining from warm flesh from which I grew,
You surely breathe and eat and cry like I.
For what can I now know of death, of that passage
That will one day threaten the incomprehensible world?
Silently they hang above me, beyond my reach, unreal . . .
Unreal at least except for eyes, mystical extensions,
Touch without fingers, motion without impact, something there,
But maybe not important, milkless, without warmth.
Still, you must have a name, a kind of category,
Something that can let me live at peace with you.
Could I but sense you in my way, some measure perhaps,
Could I but bridge the gulf that lies between,
Maybe I would do so, but how? what do I need?
A name is fine, but will it shore up liberty
From ignorance, from lack of touch, from measurement?
From measurement! What new idea now swarms within me?
Perhaps a mathematics, calculations from untouchables
Can clarify your orbit, predicting perigee perhaps or self collapse.
Unending accusations drain this dream: worthless metaphysics,
Not a method to trust, yet my heart does beat to it.
Have ancient genes bestowed on me this thrill,
Forgotten values to mankind accrued to permanence in me?
Urging explorations, voyages from this nursery,
They inflame my nerves where comfort never can.
Rattlesnakes see infrared and suffer no abject demise,
Bats somehow hear a soundless noise, compressibilities,
But all the same to me, here, not knowing, ignorant,
Wondering simply what it means to see beyond,
And if this oh-so-childish room will stand the test
Of time, when I no longer am its nexus.

D.D. Clayton, 1980

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An optimist's selection of nightmares

Peter J. Smith

A Choice of Catastrophes. By I. Asimov. Pp. 377. (Simon and Schuster: New York, 1979.) \$11.95.

IMAGINE a small black hole about the size of the average asteroid and with a mass of, say, a millionth of that of the Earth. Suppose then that this remarkable object were to enter the Solar System and score a direct hit on the Earth. What would the result be? The "mini-black hole" would tunnel its way into the planet, absorbing matter and thus growing as it went. If it had sufficient momentum to begin with, it would burn a path right through the Earth, causing enormous explosions where it entered and left. At the points of entry and departure life would stand little chance of survival, although the Earth itself would probably heal and go on as before.

But what if this mini-black hole had too low a velocity to enable it to pass through the Earth and instead became trapped in the terrestrial gravity field? It would then fall towards the planet's centre, overshoot, fall back, overshoot again, fall back again, and so on. Because of the Earth's rotation, however, the oscillation would not be along a single line but along a series of linked tracks. The mini-black hole would therefore carve out an irregular volume from the Earth's interior, growing larger as it did so and absorbing more matter with each sweep. Thus hollowed out, the Earth would probably collapse into the black hole with a diameter of 2 cm and a mass and gravitational field identical to that of the planet it had destroyed.

Fantasy? Possibility? Probability? No, yes, no — in that order. As it happens, mini-black holes, black holes of less than stellar size, have not yet been detected, although they were postulated on theoretical grounds by Stephen Hawking in 1974 and to that extent are a serious scientific possibility. The chance of one of them colliding with the Earth must be regarded as remote, however, if only because if such hole-planet collisions were common there would be far fewer bodies in space than there actually are. On the other hand, annihilation of the Earth by a mini-black hole is only one of a bewildering variety of possible catastrophes, many of them less bizarre than that, through which the human species might meet its end.

Indeed, there are so many possibilities here that in his book about them Asimov has had to design a five-fold classification system in order to make the overall picture intellectually assimilable. To begin with, then, we have "Catastrophes of the First Class", defined as changes such as to

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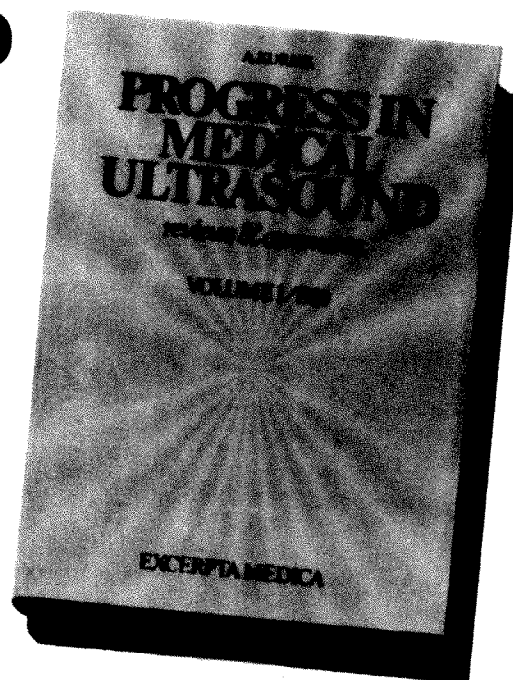
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'Progress in Medical Ultrasound, reviews & comments' will provide on an annual basis, comprehensive and critical reviews of the relevant literature found in approx. 3500 medical journals. We will do this fast and we shall try to do it accurately. We shall also speak with authority. By way of elucidation: the reader will find almost 800 references in this book to articles which have appeared in 1978 and in the first half of 1979. All reviews have been written by experts of international reknown.

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A. Kurjak

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render the whole Universe uninhabitable. One of these — the so-called heat death, by which all non-thermal forms of energy are reduced to heat, and entropy reaches its maximum — is inevitable, although it will not occur for at least a thousand billion years. Equally inevitable, though on an even less certain time-scale, is the contraction of all stellar bodies to black holes and the ultimate coalescence of the whole Universe into a single, huge black hole. And finally there is the possibility that the Universe will contract to the sort of 'cosmic egg' from which it developed via the big bang, although this depends on whether the Universe is open or closed (possibly oscillating).

Inevitable or not, however, all First Class death threats are too far ahead to worry about; they are intellectually interesting but of no practical concern. The same could be said about most "Catastrophes of the Second Class", namely, those able to destroy life on Earth by killing the Sun, leaving the rest of the Universe intact. Ultimately — in billions of years time — the Sun will develop into a red giant and then white dwarf, ending its role as supporter of life on Earth. Moreover, in the shorter (but still very long) term the Sun could be annihilated by collision with a star, a 'normal' black hole or a large body of antimatter (which may or may not exist), all of which in any case should give plenty of warning.

With "Catastrophes of the Third Class", on the other hand, a new factor enters — the possibility (but improbability) of immediate death. An undetected mini-black hole of the type mentioned earlier could even now be about to enter the Earth's atmosphere, interaction with which would give a three-minute warning of the planet's annihilation. But if that sounds alarming, it has to be admitted that most other Third Class changes, defined as those potentially able to destroy all terrestrial life by disturbing the Earth itself, are unlikely actually to do anything of the sort. Collision with extraterrestrial objects (asteroids, meteors etc.), interaction with the Moon, earthquakes, volcanoes, moving plates, glaciation and a disappearing geomagnetic field all can, and do, give rise to local disaster, and some (especially glaciation) could conceivably destroy modern Western civilization; but only in very exceptional circumstances could they obliterate all life, or even all human life.

Which brings us to "Catastrophes of the Fourth Class", or those capable of destroying all human life but not most other life forms. This category includes being overrun by insects or rodents, war, the spread of infectious disease and attack by superior extraterrestrial intelligence, although war (especially thermonuclear) and a hitherto unknown disease (perhaps man-made) seem to be the only two worth worrying about. Perhaps of rather more concern, however, are those activities and

events unlikely to destroy all human life but able, nevertheless, to ruin civilization as we know it — "Catastrophes of the Fifth Class". War again, depletion of natural resources (including energy) and pollution are the chief contenders here.

Asimov is an optimist, evidently believing that those catastrophes possible in the short term are avoidable with intelligent handling, although he warns that success in overcoming them could lead to new dangers such as overpopulation and starvation. Be that as it may, he has left us in the meantime with a remarkable survey of the possible dangers, ranging from the

fascinatingly bizarre to the frighteningly realistic. His book, though by no means short, manages to pack an astonishing number of explanatory asides (on entropy, quasars, red giants, supernovae, DNA, cosmic rays etc.) into a story covering an equally remarkable number of primary disciplines (cosmology, geology, biology, technology etc.). I could quibble a bit over points of presentation, but there is no denying Asimov's explanatory powers. □

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Theology after Darwin

A.J. Cain

The Post-Darwinian Controversies. By J.R. Moore. Pp.205. (Cambridge University Press: Cambridge, 1979.) £18.

THE subtitle of this book is "A Study of the Protestant Struggle to Come to Terms with Darwin in Great Britain and America 1870-1900", and indicates its nature far better than the main title. As such, it is not in one sense a book to review in a scientific journal, although *Nature* did publish an exchange of letters between Asa Gray and G.J. Romanes on the religious implications of evolution, in 1883. In another sense, as a study of the intellectual forces resisting, modifying, or encouraging the spread of a particular complex of ideas, it is of interest to everyone.

An idea or complex of ideas is introduced into so heavily structured a space, and engenders such complex reactions, that any account of its spread can only be made, as yet, historically, philosophically or bibliographically. I once consulted a professor of plasma physics on the mathematics necessary for modelling the spread of an idea; he dismissed the subject out of hand as "too hopelessly complicated". And as any historian, philosopher or bibliographer has himself a mind already structured, his account will necessarily be slanted to some extent. Slight slanting is insidious; blatant slanting usually produces a rival account, useful or merely eristic.

James Moore is particularly concerned to do away with the old metaphor of warfare between ideas — the victorious army of the Darwinians, the forces of obscurantism in full flight, the signal victory of Huxley over Wilberforce, and so on. It cannot be denied that there were plenty of people who belonged to neither 'army'; they accepted doctrines of both

camp, and struggled to reconcile them. Two chapters of the book are entitled "Warfare's toll in historical interpretation" and "Towards a non-violent history". These are in Part I, "Historians and Historiography", in which Moore points out the evils of such a schematic representation of extremely complex events. He blames John William Draper (*History of the Conflict between Religion and Science*, 1874) and Andrew Dickson White (*A History of the Warfare of Science with Theology in Christendom*, 1896) particularly for the prevalence of the metaphor in the evolutionary context, giving very useful sketches of their careers and intellectual conflicts to explain both the nature of their books, and the wide difference in scholarship between them. In Chapter 4, "Towards a non-violent history", he brings out his own approach, deploring the use of the military metaphor as showing "the absence of any deep moral aversion from war". Yet he himself allows (as any man of sense must) that "Christians in the late nineteenth century were beset with spiritual disorders and intellectual strife". The metaphor of armies of individuals fighting may be unsatisfactory; at this point the reader might think him about to take up that of gladiatorial combat between ideas within a single individual, which at first sight is nearer the truth. But it is far more useful, as Moore shows, to take a less superficial view. He uses Festinger's theory of the structure of cognitive conflict to show how the individual (merely the arena in a gladiatorial metaphor) is active in reducing the dissonance between incompatible ideas, either by altering one or other idea, or by introducing new ones that reduce the dissonance. He illustrates the point pleasantly by a flat-earthier confronted by an extra-terrestrial photograph of the Earth, and more seriously by the example of that unfortunate man St. George Jackson Mivart. Violence, then, is replaced by the reduction of dissonance — an undoubted gain, but one cannot help feeling that a real baby (incompatibility of ideas leading to real distress) was nearly

thrown out with a lot of bathwater.

Moore gives a useful sketch in Part II, "Darwinism and Evolutionary Thought", of the actual scientific and philosophical issues at work in his period, showing Darwin's difficulties, methodological criticisms, the influence of Herbert Spencer, and the differences that transformed Darwin's Darwinism into neo-Darwinism. (His account of the influence of Paley on Darwin, later in the book, is especially valuable.) Unfortunately, he adopts Morse Peckham's distinction of Darwinism and Darwinisticism — Christian Darwinism "understood Darwin's theory and left it substantially intact, neither emasculating it nor adulterating it with foreign ideas in the interests of dissonance reduction" and Christian Darwinisticism (unpleasing term) "either misunderstood, misinterpreted, or modified Darwin's theory, adulterating it as they had need with non-Darwinian ideas". (Surprisingly, these definitions are not in the index.) It comes as a shock to the student of evolution to find Lamarckism labelled Darwinisticism.

Lastly, in Part III, "Theology and Evolution", Moore makes his most valuable contribution, with sketches of 28 Christian controversialists, American and English, their intellectual predispositions, development and final attitudes. Most are substantial figures, well worth analysing. A few are more reminiscent of Elderess Polly, Elderess Antoinette and Newman Weekes, in Matthew Arnold's bland and devastating account of religion in America. Moore shows that some more orthodox Christians, especially Calvinists, had far less difficulty in accepting natural selection and the struggle for existence as the true cause of evolution than did most liberals, and that much of what has been written on the period shows a complete lack of understanding of Protestant stances.

It would require a far more massive exposition even than Moore's to do justice to these great themes. Moore has much to say on progress, providence and criteria of explanation. Yet his treatment of theological themes omits, rather surprisingly, all useful mention of the Fall, and not too much is said of the creation of Adam and Eve. The theologically orthodox positions he discusses (and the reader is sometimes not clear about *which* orthodoxy he is discussing) are mainly of Dissent; Catholics and Anglicans feature prominently but are not as well analysed as Congregationalists, Unitarians or Presbyterians. A more serious weakness is superficiality in the analysis of some of his characters. For example, Frederick Temple is commended (rightly) for his "generous and incisive" sermon to the British Association the day after the famous encounter of Huxley and Wilberforce, and his *Relations Between Religion and Science* (1885) is quoted as reducing natural selection to "one partial expression" of the

original properties impressed on matter by the Creator. (This, of course, is the old fallacy that if you can write an equation for something, then the equation being devoid of emotions, so should we be in contemplating the thing.) Moore also quotes Aubrey Moore's correct criticism that Temple's attitude although he was an Anglican clergyman (and later Archbishop of Canterbury) was pure Deism, not Christianity. But he does not point out that Temple's *Relations* is one of the worst examples published of using the then ignorance on certain scientific subjects to insist that God must have acted directly in these matters. Huxley's correct criticisms of Lord Kelvin on the age of the Earth disproving evolution by natural selection

are dismissed in quoted words as to be "praised more for their vigor than their strength", although a divine making the same point is referred to without qualification. (It is not always clear whether the book is an analysis of attitudes, in which case it should be more trenchant, or a demography, which requires a greater coverage of people.)

This book is a 'must' for historians of ideas, useful for students of evolution and theology, and quite interesting enough to recommend to the public generally. □

A. J. Cain is Derby Professor of Zoology, University of Liverpool, UK.

Better buy a pair of climbing boots

Fred Dainton

Scientific Productivity: The Effectiveness of Research Groups in Six Countries. Edited by F. M. Andrews. Pp.469. (Cambridge University Press: Cambridge, UK, and New York; UNESCO: Paris, 1979.) £20.

REMINDED by the title of this book of currently fashionable productivity deals in wage bargaining and on the evidence of a photostat of the title page, the list of comments and the editor's explanation of the purpose of this book, I undertook to myself to review it in a matter of two or three weeks even though I have a rule that all reviewers have an inescapable duty to read every word the author puts before them. I failed in the task not because of any physiological inability to reproduce on my retinas faithful images of the words in front of me nor because of any lack of interest in the subject (wouldn't we all like some philosopher's stone which would enable us to improve the productivity of research units?), nor because of any unwillingness to learn the techniques of the sociologist, so abundantly deployed here, if that were the necessary price of wisdom, but simply because of a great weariness of the flesh induced by perusal of the pages.

As I hacked my way through the verbal undergrowth, pausing to absorb the significance of each of the numerous qualifying clauses, worked my way through complicated diagrams and then re-read in order to make sure that despite all appearances there must be gold somewhere, I longed for the experience, common sense and humanity of a Medawar to tell us in plain words how

scientific productivity can be increased. My prayers were answered for on the 28th February his *Advice to a Young Scientist* was published and happily fell into my hands, and, though this latter book contains no figures, no tables and less than one-fifth of the verbiage of *Scientific Productivity* and is not explicitly directed to this subject, Medawar has far more of value to say to scientists, scientific administrators, and science policy makers and watchers at one-quarter of the cost.

Scientific productivity is a difficult concept, raising questions of volume, intellectual or experimental excellence, magnitude and nature of the impact of the research unit's output on the development of the subject (some developments seen as exciting at the time they are published are later shown to be inhibitors rather than catalysts of progress, a point not really brought out in the book), applicability to economic or social ends etc., etc. Moreover the weight to be attached to each of these many factors might be expected to depend not only on time but on the social and political viewpoint of the assessor. So one has great sympathy with the team of investigators in their methodological difficulties. Therefore the reader tends to concentrate on the conclusions to see if they are so much better and more useful than widely held views and opinions that, as that rock of common sense Ralph Waldo Emerson predicted "The world will make a beaten path to his [in this case the authors'] door". I fear the world will not, for who will want to read so much to arrive at conclusions like the following. I quote from Part 1: "The social position of the individual within the social hierarchy of a research unit proves to be one important correlate of differences in performance at the individual level and the size, age and scientific exchanges of the research unit are additional factors that relate to group productivity", "The results from academic research units seem to be in accord with the 'human relations thesis' that is, that the idea that good leadership

leads to a high group morale and that high morale leads to increased productivity by group members", "that human resources are more significant than [financial resources or access to information]", "The results show that higher levels of effectiveness tend to occur where there is more communication" and "That higher performing units tended to have more dedicated professional members with more diverse working roles and intellectual resources".

But one must have some sympathy for the authors. It seems that a questionnaire, reproduced in an appendix, was sent out to a large number of workers in many European research units. 11,000 of them in 1,200 units distributed over six countries responded. Those who devised the questionnaire are also the authors of the 14 chapters which comprise the book and they had at their disposal all the information elicited by the questionnaires. Apart from the first two chapters (in many ways the best in the book) describing how the International Comparative Study on the Organisation and Performance of Research Units was conceived and mounted and the resultant data analysed, each chapter is by a different author or group of authors. Full freedom was given to each to use his or her own methodology and to exercise personal judgements, and no attempt was made to draw them into a symposium at which differences and common features of their conclusions could be collectively explored. Indeed there seems to have been a perverse pride in avoiding this useful device and also in inexplicably arranging the chapters in an order "alphabetical by the authors' national or international affiliation". I expect that, like myself, other sociologically illiterate scientists would have been grateful to have had some guidance provided by a consensus of the consortium members as to the weights to be attached to the various findings. Nonetheless several chapters do have some useful reviews of past work, and some conclusions are drawn from this and from the empirical data yielded by the enquiry.

It is sadly ironic that the book appears under the imprimatur of Cambridge University where so many highly successful research units have been established either by administrative fiat or more often have evolved from a happy coincidence in time and place of an opportunity and those capable of seizing it. This prompts the questions as to whether Max Perutz, when building up and running the highly successful Laboratory of Molecular Biology in Cambridge, and indeed others with similar track records, would have done better if they had had all these conclusions firmly embedded in their consciousness and, more importantly, whether those charged with the responsibility for establishing units in the future would make better decisions after reading this book. The answers must

remain a matter for conjecture but I am quite certain that they would have been even better advised to read Medawar and then ask themselves a series of questions: for example "Would a research unit in this field offer a reasonable prospect either of a significant advance in knowledge or of meeting some societal need? How can I identify and appoint the highest quality person(s) to lead it and procure the necessary material resources for them? What is the best intellectual environment for the unit to flourish?" And perhaps of greater importance "How can the inevitable tension between the paymaster and the research unit be mediated so as to maximize the freedom of the Director and his staff which is necessary for high creativity and productivity without giving them a licence grossly to ignore the terms of reference under which they operate?" Then there is finally the most difficult question as to how one is to know when the research unit has served its purpose or has lost its quality and should be closed. There is little in this book which helps with the resolution of these practical problems and what there is is difficult to quarry without reading the whole book for there is no subject index.

Of course this is not the only book which fails such tests of practical utility to the decision makers but which may nonetheless be of value to the community of scholars to which the authors belong, in this case all those interested in sociological enquiry. I am not competent to express any opinion on this latter question, and in any case this is perhaps of less relevance because the Foreword by UNESCO makes it plain that the work was the outcome of that body's Nineteenth General Conference and the same Foreword refers sympathetically to "... citizens in many countries asking for more public accountability. They want to know the use to which their resources are put and they demand that the programs that they support shall be both efficient and effective". That these desires should be gratified is unexceptionable. This principle can equally be applied to the UNESCO programme of which this book is the visible and ponderous product. All the more pity that we are not told how much the whole enterprise cost and thus be able to judge whether the expenditure was justified. I have a strong feeling that if those scientific directors of research units who spend £20 on this book were each to buy, at slightly greater cost, a pair of good mountaineering boots and, after salubrious daily scrambles, to confer on this topic in the evenings throughout a week spent at some delectable Alpine centre they would bring down from the mountain more useful tablets than this book. □

Sir Fred Dainton is Chairman of the British Library Board, London, UK.

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Coats of many colours

Anne McLaren

The Coat Colors of Mice. By W.K. Silvers. Pp. 379. (Springer: Berlin, Heidelberg and New York, 1979.) DM59.60, \$32.80.

SOME targets of scientific study are inherently attractive. One such is soap bubbles. Another is coat colours and patterns. In the days when I knew R.A. Fisher, the father of modern statistics, his true pride and love was for mouse genetics, and to be asked the effect of umbrous on an agouti pink-eyed brown background would really make his eyes twinkle.

W.K. Silvers' book is also a labour of love. There is no mention of Sturtevant's intriguing suggestion that Mendel discovered his 'laws' of segregation and assortment by illicitly crossing different-coloured mice in his monastery cell, later using his curiously exact pea ratios as a mere didactic demonstration; but in other respects the early history of the subject is well covered. The references include more than 20 papers published in the first two decades of this century on the genetics of coat colour in mice, starting with the classic 1902 paper of Cuénot. One of these pioneers is still living, Naomi Mitchison, who, with her brother J.B.S. Haldane, published in 1915 the linkage between albino and pink-eyed dilution, the first to be established in any mammal. A start was also made in those early years on the chemistry of mouse pigmentation (Onslow, 1915) and on the developmental analysis of lethal yellow (Ibsen and Steigleder, 1917), while C.C. Little's life-long interest in mouse spotting genes gave rise to a series of papers beginning in 1913.

Moving to more modern times, Silvers pays tribute to Grüneberg's monumental *Genetics of the Mouse* (Nijhoff: The Hague, 2nd edition; 1952), to Searle's *Comparative Genetics of Coat Colour in Mammals* (Logos Press: London; 1968), to Mintz's illuminating studies on experimental chimeras ('allophenic' mice) and to the great amount of assistance that he received from Rita Phillips, whose very recent death is a sad loss to mouse genetics as well as to her many friends and colleagues.

Apart from its aesthetic appeal, the study of coat colours embraces not only genetics (148 alleles at 63 loci), but also biochemistry, cytology, histology, anatomy, embryology and endocrinology. Silvers does justice to all these aspects. The first half of the book, in which he deals with the various colour-controlling loci and their complex interactions, is perhaps more satisfactory than the second half, which covers spotting genes, chimeras and X-chromosome mosaics. The etiology of

spotting and coat colour pattern is still widely debated, and even the experimental evidence is sometimes conflicting. Silvers does his best to summarize the literature, but the picture that emerges is patchy in more senses than one. For example, in the field with which I am most familiar, he perpetuates confusion by failing to make clear that Mintz's 'standard' or archetypal pattern is a valuable abstraction rather than an observed pattern ('observed relatively rarely' is misleading when the true probability, for one side of the mouse alone, is 2^{-16} , less than 1 in 100,000); he does not mention Lyon's interesting suggestion that the frequent brindled sectors of chimeras arise because each territory may be colonized, not by one but by two 'primordial melanoblasts', of the same or contrasting genotype; and he affords the same status to the clonal theory of pattern formation for hair follicle chimeras as for melanocyte chimeras, though the evidence is far less cogent for the former than for the latter.

But these are minor grumbles. The book as a whole will be a standard reference work in every mouse genetics laboratory for many years to come. Not only does it make admirably clear the complex interplay of genetic (and in some cases environ-

mental) factors in determining coat colour, but it also uncovers areas where further research is badly needed. These include spotting genes, of course; the intriguing behaviour of silver, resembling the effects on pigmentation of irradiation, and perhaps also ageing; the 'reds' of the mouse fancy, and no doubt other fancy types also, not yet analysed genetically; and, above all, the need for a codification of colour, a decent set of colour standards, which although doubtless imperfect could hardly but improve on such phrases as "a dull brown colour", "a medium shade of sepia", "a faint cream or ivory color", "a brownish shade, a little lighter than an ordinary pink-eyed brown with a slight dull yellowish cast". The three colour plates in the present volume are not of very high quality, though better than nothing; really good colour reproduction would have presumably escalated the price unacceptably.

As it is, the book at about £15 is reasonably priced, there are good author and subject indexes, and the bibliography is comprehensive. □

Anne McLaren is Director of the MRC Mammalian Development Unit, London, UK.

The classic electricians

W.D. Hackmann

Electricity in the 17th and 18th Centuries. By J. L. Heilbron. Pp. 606. (University of California Press: Berkeley, Los Angeles and London, 1979.) £24.

AN ambitious work that deserves to become a classic in this subject. Not only has Professor Heilbron incorporated all the historical papers on early electricity that have appeared since I. B. Cohen's *Franklin and Newton An Inquiry into Speculative Newtonian Experimental Science and Franklin's Work in Electricity as an Example Thereof* (The American Philosophical Society: Philadelphia, 1956), but he has also included much new material, such as the importance of the seventeenth century Jesuit polymaths in the study of physics in general, and in electricity in particular. For those of us reasonably well versed in this subject, the main developments as given in this book are known, but it is useful to have such a broad and up-to-date survey. The general reader interested in the history of physics, or of electricity, should find this a fascinating story, although he might miss some of the finer philosophical or technical nuances. Any historical treatment of a scientific subject must contain inevitable

distortions as all is focused on specific aspects of the total picture, but by attempting such a broad canvas, Heilbron has managed to keep these distortions to a minimum.

A work of this nature, covering such a large time span, is inevitably faced with historiographical problems. Terms and concepts change their meaning, brought about by new discoveries and by changes in philosophical outlook, and this makes interpretation difficult. The author has to make it clear to the reader that a problem dealt with in the seventeenth century was handled differently from the same problem looked at by physicists one hundred years later. When surveying a subject over a very long period, it is possible to give it a false appearance of continuity, while the subject progressed (or changed) in a much more convoluted manner. In the context of this book, both the concepts of physics and of electricity changed dramatically during these two hundred years. The word 'electricity' was to undergo subtle changes in meaning during the century after its use by Sir Thomas Browne in his *Pseudodoxia Epidemica: or, Enquiries into very many received tenents and commonly presumed truths* (1646), causing endless confusion to historians of science in the past. At first it simply referred to the attractive property of amber and other substances. The word only began to denote a separate entity or substance causing the electrical behaviour of attraction and repulsion during the early 1740s. Heilbron has coped admirably with this kind of problem, not least by the way

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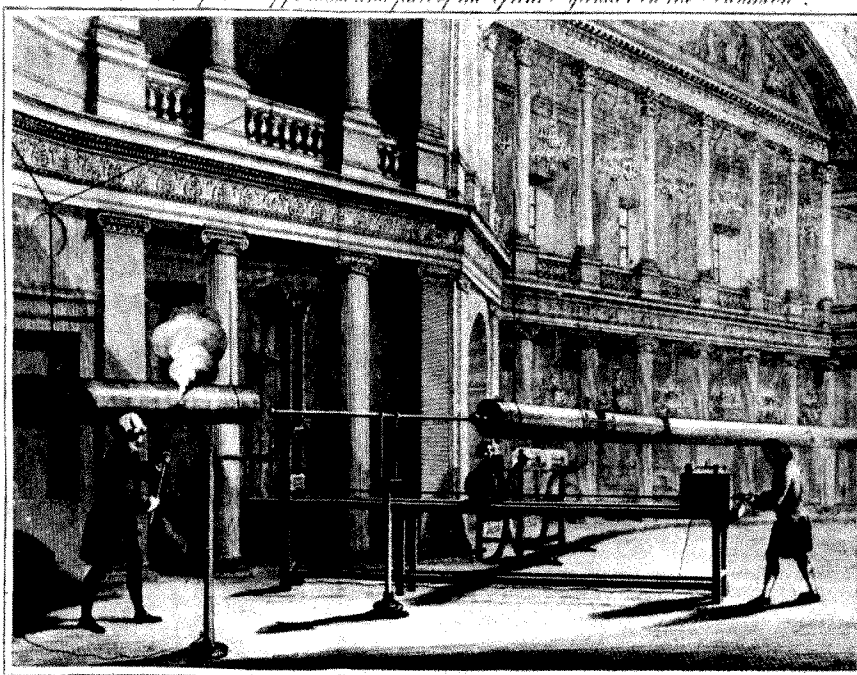
he has structured this book, which he has divided into five parts.

Part 1 sets the scene by giving a general survey of early modern physics, its underlying principles and the ways in which it was diffused, including the founding of scientific societies, the development of better instruments and the importance of lecture-demonstrations. He divides some 210 electricians (as those studying this subject called themselves) into five groups according to their chief means of support: members of religious orders (especially Jesuits), paid academicians, professors, public lecturers, and others (primarily instrument-makers, doctors, lawyers, clergy and the independently wealthy). Between one-quarter and one-half were professors of universities or secondary schools. It is difficult to determine how approximate these figures are. They contain overt generalizations (of which the author is aware), and there are inevitably minor inconsistencies and errors. For instance, in the table dealing with the number of academies founded between 1660 and 1800, he lists no provincial societies under Britain (apart from one by name), while under 'others' in the case of France, he lists 27, and under Holland 14 (these include agricultural societies). This produces a distorted and not very meaningful picture. In general, however, his conclusions are justified.

Part 2 surveys the progress of electricity during the seventeenth century, starting with Gilbert's experiments on the amber effect in *De magnet* (1600), terminating soon after Otto von Guericke's experiments with his 'globus mineralis' in the 1660s. Part 3 covers the first half of the eighteenth century, starting with the researches of Hauksbee the elder, at the Royal Society during the early 1700s. Part 4 deals with the impact of the Leyden jar (a primitive condenser), discovered in 1745, and the reception of the theories of Benjamin Franklin. Part 5 covers the last thirty years or so of the eighteenth century, during which the main electrostatic principles were finally formulated and attempts were made at quantification. This period is terminated with an Epilogue describing the invention of the voltaic pile or battery in 1799. During the entire two hundred years, 'electricity' meant electrostatics, then known as 'frictional electricity', as it dealt with charges generated by rubbing glass or other electrics.

The sub-title, "A Study of Early Modern Physics", is significant, for the author has tried to relate the history of electricity to the broader conceptual changes that occurred during the rise of early modern physics. Effectively, he has divided the history of this subject into three stages. The first stage, which lasted until 1700, was still dominated by Aristotelian physics, a textbook science which included organic and psychological as well as inorganic phenomena. The second stage, lasting until

Wilson's public model lightning conductor experiments at the Pantheon, London, 1777.



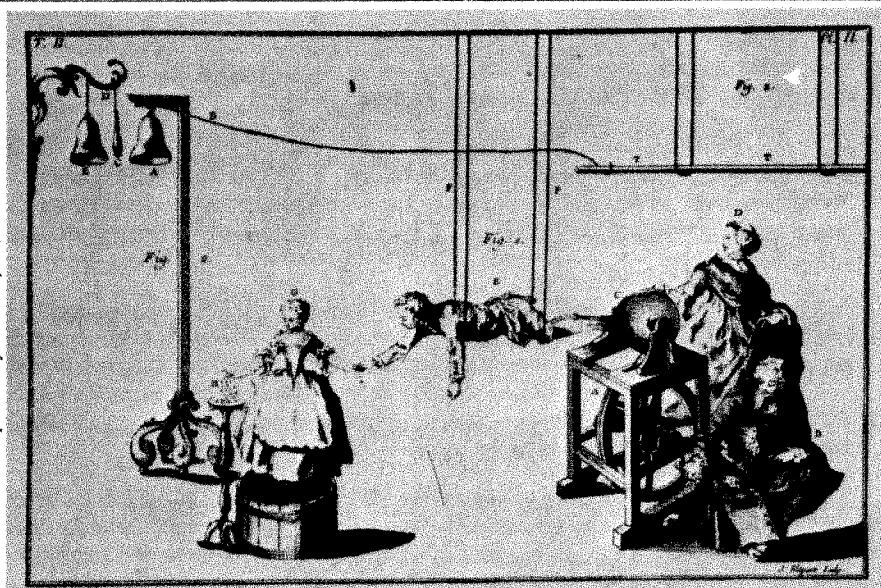
the 1750s was dominated by the followers first of Descartes, and then of Newton. The Newtonians, who by their virulent empiricism had taken Newton's "hypotheses non fingo" much further than had been intended by their master, gained the upper hand during the 1720s. Physics took on a more restrictive and modern meaning; the subject now being restricted to astronomy and experimental physics (alternatively known by the vague English term of natural philosophy), which included mechanics, hydrostatics, pneumatics, optics, electricity and magnetism, but excluded subjects such as psychology, medicine and the biological sciences. The importance of applied mathematics in the formulation of physical laws began to be stressed, amongst others, by 's Gravesande, but this approach only gained momentum during the third and final stage. Heilbron points out that the advances made in quantifying electrical phenomena from the 1760s onwards owed nothing to the progress of mathematics. Indeed, not until the turn of the nineteenth century did electricians require mathematical techniques not fully available a hundred years earlier.

In order to make progress in electricity, required were (1) carefully worked out experimental procedures which could be replicated by different physicists, (2) accurate and reliable instruments, and (3) the isolation from the mass of experimental details of those factors that were important in producing the observed phenomena. Heilbron's narrative demonstrates how the conflicting data took many years to untangle. These three requirements are, of course, interrelated. Nature was often 'manipulated' in those areas in which new phenomena were expected. Attempts to store charge and to electrify water resulted in the discovery of the Leyden jar by von

Kleist in Germany, and Cunaeus and Van Musschenbroek in Holland, although the results were unexpected and went far beyond the experimenters' expectations. Incidentally, Heilbron honours Cunaeus with this invention, but this claim cannot be substantiated. Simultaneous and independent discoveries of this nature usually signify that the conceptual framework and experimental techniques have become available for these breakthroughs to be made.

Heilbron is fully aware of the importance of the Leyden jar. It led to the study of more powerful electrical phenomena, and it assisted with the formulation of new concepts such as that of electric circuit, intensity or level of electrification (an anticipation of potential), capacity or area of coated surface electrified, and quantity of charge, for it could readily be compared with the behaviour of liquids flowing from vessels of different sizes. The invention of the electrophorus (1775) and the parallel plate condenser (1778), based on the Franklinian explanation of the Leyden jar, not only strengthened the formulation of these concepts, but also focused on minute electrical charges (Volta's 'contact electricity'), which eventually led to the invention of the voltaic pile. Early attempts to explain this remarkable device in terms of standard electrostatic theory based on the Leyden jar failed, and it did not take long for scientists to realize that other concepts such as current intensity and electrical resistance were necessary. Thus, instruments influenced the formulation of new concepts, and the latter in turn affected instrument design. Although his conclusions are broadly in agreement with this view, Heilbron's general discussion on the development of electrostatic instruments is weak, and contains minor factual

Globe machine with suspended boy conductor, 1748.



errors.

Quite properly, Heilbron devotes an entire chapter to electrometry. He discusses the key electrometers, especially those of Robison (1770s) and Coulomb (1784). One does not, however, become aware of the existence of the great number and diversity of electrometers invented during the final decades of the eighteenth century, caused by the search for

replicability of measurements. Attempts to standardize measuring techniques and introduce electrometric standards prior to Coulomb's work failed, mainly because there was no proper understanding of what the different electrometers were actually measuring. According to Heilbron, what was lacking was the appreciation between the difference of electrostatic force at a macroscopic level (moving force or kinetic

energy), and an (idealized) microscopic force as an unobservable push or pull between elements of electrostatic charge. The triumph of Newton's theory of gravitation was to compute force by summing forces, but this distinction was not generally understood by physicists before the second half of the eighteenth century. Modern historians of science, too, have not always made this distinction when interpreting the early attempts by Bernoulli (1760) and others to relate the behaviour of electrostatic force to the inverse-square law of gravitation. Robison, Cavendish and Coulomb were the first to achieve this. They were the vanguard of a new breed of physicist, unlike their predecessors more interested in exact mathematical descriptions than in the qualitative models which had played such an important role in the early history of electricity. They formed the bridge between the qualitative work of the preceding two hundred years and the mathematical synthesis by Poisson and others in the nineteenth century. □

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Plasmid horizons

Naomi Datta

Plasmids. By Paul Broda. Pp.197. (Freeman: Oxford and San Francisco, 1979.) £6.90, \$18.50.

RESEARCH on plasmids is exciting and varied. It includes intensive study of very basic biological functions such as DNA replication. F-mediated conjugation provides an example of differentiation of structure and function in a single-celled organism, subjected to detailed genetic analysis. Insertion sequences and transposons are newly discovered and evidently important means of genetic variation allowing evolutionary changes in response to changing environment. Plasmid-determined antibiotic resistance and determinants affecting bacterial virulence are of great medical relevance, and study of the latter, in particular, may lead to new insights into bacterial pathogenicity. Plasmid synthetic and degradative enzyme systems, evolving all the time, are likely to be of increasing commercial and environmental importance. And, as well as all these reasons for the study of plasmids as they exist in nature, their use as vectors in genetic engineering opens up new horizons in biological research both academic and pragmatic. No wonder that the number of papers published on plasmids seems to be increasing exponentially. An up-to-date, comprehensive and comprehensible guide to the subject was badly needed by

undergraduates or research workers starting out in this field. Here it is — Paul Broda has provided just the thing, and it is not only newcomers to the subject who will welcome his *Plasmids*. Plasmid research has its own specializations, broadly on the lines of the subject-matter of the different chapters of this book, and people knowledgeable about any one of them cannot hope to keep up with all the others.

This is a short book, only 148 pages of text excluding references and index, and I was surprised at the amount and variety of detail packed into such a small space. The layout is organized imaginatively to assist a reader to grasp principles with which he may not be previously familiar. The many summarizing tables inserted in the text are particularly successful both as teaching aids and for quick reference. After a brief introduction to define the subject and sketch in its short history, we are presented in a thoroughly practical way with the methods used for isolating plasmids from their host cells. These methods are of two main kinds, either on a fairly large scale to obtain plasmid DNA for further study, or rapid and small-scale, adaptable to many experimental purposes. Great advances in methods of both these kinds have been made in the last few years. It is appropriate that this section should come early on in the book since much of the information on plasmids in later chapters has resulted from the use of these methods.

The chapter on the structure and evolution of plasmids illustrates how those creatures have developed by connecting as well as by diverging evolutionary paths. The use of endonucleases is described, in the characterization of plasmid DNA and also for the construction, *in vitro*, of new plasmid molecules. This implies a very broad meaning for 'evolution', but why not?

The complex subject of DNA replication is considered in Chapter 4. Plasmids are more accessible to experimental manipulation than are whole cells and therefore plasmid replication has been studied, not only for its own sake, but also to elucidate host mechanisms for, and controls over, DNA replication. This chapter covers both plasmid-determined and host functions and throws considerable light on a confusing area of research. Here, as elsewhere in the book, the limitations of current knowledge are pointed out. It is a comfort, sometimes, to be told that something is still unknown.

Transfer of DNA by conjugation in bacteria is, as far as is known, always plasmid-determined. The chapter on the subject summarizes the results of detailed investigations into the conjugative functions of F and its relations, and indicates how little is known about conjugation determined by many other, unrelated, plasmids. It is possible that the production of pili is a prerequisite for conjugation despite the instance, cited on p. 97, where they were apparently not essential. This case referred to plasmids of

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Michael M. Benarie

Head of the Department of Air Pollution, National Institute for Chemical Research, France.

Summer 1980; £30; 284pp; ISBN 0 333 23391 3

This monograph collects together the methods, models and formulae used for estimating air pollutant concentrations in urban areas. The purpose of this work is to assess the aims and the usefulness of the available methods and to show that urban modelling is not just a gaussian plume, but a combination of complementary approaches and possibilities.

READERSHIP: Civil engineers, meteorologists, urban planners and administrators, environmentalists.
LEVEL: P, G, U.

The Fast Breeder Reactor. Need? Cost? Risk?

edited by **Colin Sweet**

Senior Lecturer in Economics at the Polytechnic of the South Bank.

Summer 1980; £17; 208pp; ISBN 0 333 27973 5

This work explores some of the major implications of fast breeder technology. The energy policy relating to nuclear power and the possible alternatives to nuclear energy are discussed in the first section followed by an appraisal of the risks involved in fast breeder technology. The economics of nuclear power and coal are compared and the various options that nuclear power offers for the future are discussed. An interesting section on nuclear power and civil liberties is also included.

READERSHIP: Engineers and scientists involved in energy planning, economists, general readers interested in the 'nuclear debate'.
LEVEL: P, G, U, GR.

Cardiovascular System

Edited by **R. Shanks**

Professor Clinical Pharmacology, Queen's University, Belfast.

Summer 1980; £10; 106pp; ISBN 0 333 28604 9

This work contains a selection of articles first published as a monthly series in The British Journal of Clinical Pharmacology. The articles describe the main methods for the investigation of the effects of drugs on the cardiovascular system in man. All the main aspects of the cardiovascular system are covered including cardiac function and the peripheral circulation with separate chapters being devoted to different methods for examining the same basic function.

READERSHIP: Pharmacologists and clinical pharmacologists, cardiologists.
LEVEL: P, G, UR.

The use of alternatives in Drug Research

Edited by **Andrew Rowan**

Assistant Director, Institute for the Study of Animal Problems, Washington DC and Carl Stratmann, formerly Scientific Consultant for the Fund for the Replacement of Animals in Medical Research (FRAME).

Spring 1980; £8.50; 224pp; ISBN 0 333 27014 2

This volume, based upon a symposium organised by FRAME, has been prepared to consider some of the alternatives to animal use in the specific field of drug research. The eleven papers presented here range over many aspects of the issue. Taken together they provide a valuable focus for discussion and by identifying specific topics for future research this work should help to lead to a greatly reduced use of animals in drug research.

READERSHIP: All research workers in the Biomedical Sciences who undertake any experimentation on animals using drugs.
LEVEL: P, G, UR.

Enzyme Inhibitors as Drugs

Edited by **Merton Sandler**

Professor of Chemical Pathology, Bernhard Memorial Research Laboratories.

Summer 1980; £25; 250pp; ISBN 0 333 28984 6

A new volume in the Biological Council's Drug Action Symposia series, this work covers the developing links between enzymology and pharmacology and the rapidly expanding new field in pharmacology which results from these links. The contributors are leading international authorities in this field and the topics covered included the key subjects of research and debate.

READERSHIP: Enzymologists, immunologists, and pharmacologists.
LEVEL: P, G, UR.

KEY: P = professional; G = graduate; U = undergraduate; UR = undergraduate reference; GR = general readers.
For further details contact Frances Roach, Scientific & Medical Division, Macmillan Publishers, Houndmills, Basingstoke, Hampshire RG21 2XS.

group N whose pili have only recently been seen (Bradley, *Plasmid 2*, 632-636, 1979). The chapter on plasmids in human and veterinary medicine gives an overview of the astonishing evolutionary phenomenon of antibiotic resistance. Very often, within a few years of the introduction of a new antibacterial drug, bacteria resistant to that drug have appeared, the resistance being plasmid-determined. The author points out (p. 138) that the molecular events leading to the appearance of new resistance plasmids may be rare, the intensity of selection for resistance making their effects quickly evident. Gentamicin resistance, for example, is already more frequent in both Gram-negative and Gram-positive bacteria than it was at the time of publication of the papers cited on pp.114-115 of this book, a result of the widespread use of gentamicin in hospitals.

The same chapter also reviews what is known of plasmids that confer pathogenicity. There is reason to hope that research on naturally occurring plasmids, and on plasmids used as cloning vehicles for 'pathogenicity' genes, will extend our present very limited understanding of the effects of bacterial infections.

A chapter entitled "Other Plasmids" describes those determining colicinogeny, degradative functions in pseudomonas, plant tumour causation by *Agrobacterium tumefaciens*, and antibiotic production and resistance in actinomycetes. Obviously, in such a short space there must be much abbreviation and some simplification, and indeed this applies to the whole book. The references, however, are well chosen (the method of choice is explained in the preface) and numerous enough to help the reader to find out more about any of the topics covered in the text.

I have not found many errors in this book and those I have detected are fairly trivial. (To give one example, the semi-synthetic penicillins resistant to staphylococcal penicillinase are described as "cloxacillins" — they should be called isoxazolyl penicillins, of which cloxacillin is one example.) Also, considering the size of the book, I have found very few omissions. The only mention of plasmids in strictly anaerobic bacteria is that referring to *Bacteroides ochraceus* (p. 139); plasmids in *Clostridium* species as well as bacteroides might have been mentioned in Chapter 6.

Two previous single-author books on plasmids are *Bacterial Plasmids* by G. G. Meynell (Macmillan, 1972) and *Infectious Multiple Drug Resistance* by S. Falkow (Pion, 1975). Both are good books but neither, in 1980, can provide for the readers who will, I believe, gratefully welcome Broda's book. □

Towards enlightenment in radioactivity

Thaddeus J. Trenn

Radioactivity in America: Growth and Decay of a Science. By Lawrence Badash. Pp. 327. (Johns Hopkins University Press: Baltimore and London, UK, 1979.) £11.50.

THE value of history may well lie in the perspective the past can provide for the present in its relation to the future. Radioactivity is often talked around today, but generally with little understanding. Radioactive rain and snow were present long before such phenomena were discovered and investigated scientifically. Primitive man had a great fear of lightning and other forces of nature, yet with respect to radioactivity most of us still stand today as did the Neanderthals. Knowledge is power but ignorance breeds impotence. The first step to correct this source of insecurity and fear is to learn what makes it 'tick'. To this end it would be valuable to trace the historical development of man's understanding of radioactive phenomena in a manner accessible to the layman. This is what the book under review has done and why it is so important.

The science of radioactivity had a long and complex history, starting with Becquerel's discovery of natural radioactivity in 1896 and reaching to the discovery of artificial radioactivity by Joliot and Curie in 1934. A spate of major tomes, from about 1930, attests to the maturation of this multi-faceted discipline: Meyer and Schweidler, *Radioaktivität* (1927), Kohlrausch, *Radioaktivität* (1928), Rutherford, Chadwick and Ellis, *Radiations from Radioactive Substances* (1930), Curie, *Radioactivité* (1935), and regular reports on the subject which appeared until 1943 published in the *Annual Reports on the Progress of Chemistry*.

Badash narrows the subject both in time and in range by focusing upon what radioactivity research there was in America from scientists such as Boltwood, McCoy, Baskerville, Bumstead and Pegram. Rutherford and Soddy naturally play a central role not only because they were in Canada when they provided the theoretical explanation of radioactivity in 1902/3 — see *Nature* 274, 723-724; 1978 — but also because they attracted many research workers. Indeed, part of the decline of this American scientific specialty can be attributed, as Badash suggests, to the transfer of these two scientists to the other side of the Atlantic (Chapter 18).

Much of radioactivity research in America was directed towards a clarification of the genetic connections within the several radioactive decay sequences. Such radiochemical analysis fell

in between physics and traditional chemistry and could never really establish itself in America. According to Badash, this scientific specialty was also "suicidally successful" (p. 152); it was no longer viable once the genetic connections had been sorted out in uranium, thorium and actinium. The main lines of radioactivity research thus remained in Cambridge, Paris, Rome and Vienna, leading to artificial transmutation and artificial radioactivity. Even radiochemistry took on a new life as radioactivation analysis (see Broda, *Advances in Radiochemistry*, Cambridge University Press; 1950). In retrospect, we can mark the transition from radioactivity to nuclear physics and nuclear chemistry with the discovery of the neutron in 1932 followed soon after by Fermi's explanation of β -decay.

Badash does not always restrict his historical account of radioactivity to the scientific specialty which had its "growth and decay" in America. When dealing with the concept of isotopy and the group displacement laws, for example, Badash leaves the American scene for two full chapters with the centre of action in Europe. He is overtly sympathetic with Fajan's electrochemical approach, an alternative which phenomenologically did yield nearly the complete and correct formal scheme, although it was unsound (p.211) and accordingly lacked the explanatory power of Soddy's subsequent analysis, for which he alone received the Nobel Prize.

The decline of radioactivity research in America by the end of the Great War was conditioned by factors besides the loss of Rutherford to Europe, the lack of any institutional framework and the self-fulfilment of the goals of this scientific specialty. Radioactivity research "shared the same . . . poverty as other branches of science" in America (p.271). Therefore, what Badash has termed the "decay of a science" is perhaps simply the decline of a scientific sub-discipline as but one more manifestation of a general atrophy or perhaps the "immaturity" (p.274) of American science needing the fertilizing immigration of the 1930s to develop.

The reader may consider much of this somewhat remote from the current pressing concerns about nuclear reactors and atomic bombs. Nevertheless, all this began with radioactivity; the emission of radioactive radiations and the self-transmutation of one isotope to another being nuclear processes. That nuclei can also fission into larger fractions, and that under certain conditions this fission process can lead to a chain reaction are also natural phenomena that need to be better understood. Even scientists were surprised to learn recently that our technological 'eighth wonder' — the controlled fission reactor — had been anticipated by nature when dinosaurs still roamed the earth. Inherent checks and balances prevented this natural reactor from exploding like an

atomic bomb, since the critical state, once reached, tends to turn itself off. The very heat generated by the onset of an uncontrolled chain reaction tends to liquefy the dense and compact solid substance dispersing it, perforce eliminating the concentrated condition so essential for the onset and continuity of the critical state within a mass of fissile material.

Only if a chain reaction can be achieved and executed nearly instantaneously by intentionally overcoming the barriers nature has set can the 'atomic-bomb' effect be realized. The concentration of fissionable material in a nuclear reactor is not appropriate for this effect. In so-called thermal reactors using the isotope U-235, fissile to *slow* neutrons (neutrons of relatively low energy which have been 'moderated' to 'thermal' velocities), the chain reaction of the nearly instantaneous type required for the 'atomic-bomb' effect simply cannot occur. For the same reason, an atomic bomb consisting solely of the rare isotope U-235 would only fizzle. It is

natural uranium, U-238, which provides the main contribution to the 'atomic-bomb' effect, since it can be fissioned with neutrons which are *fast* enough to yield a chain reaction having the required celerity. Once these and other consequences of nature's inherent control mechanisms become better known, there will be less public obsession with the alleged dangerous proliferation in the supply of U-235 and allied (*slow*-neutron) fissile substances like plutonium.

Yet, to have expected the present work to deal with the totality of our misunderstanding about radioactivity and its nuclear progeny would be unreasonable. The scales can be removed from the public perception only in gradual stages. That Badash has taken us a significant step forward is perhaps a sufficient achievement.

Thaddeus J. Trenn is affiliated to the Max-Planck-Institute in Munich, FRG.

Astronomical oddities

Paul Murdin

Monsters in the Sky. By Paolo Maffei. Translated by Mirella and Riccardo Giacconi. Pp.241. (MIT: Cambridge, Massachusetts, and London, UK, 1980.) \$15.

THE monsters in the title of this book are not constellation figures, inhabitants of UFOs, dragons or other overtly mythical creatures. Monsters is used in the same sense as in the section of the *Thesaurus* called Unconformity, where it is associated with wonder, curiosity, missing link and queer fish. The "monsters in the sky" are thus unusual astronomical objects. They are the objects, says Paolo Maffei, which were better left out of his previous book, *Beyond the Moon*, on the more straightforward parts of astronomy, because they were so unusual that they were confusing. The present book assumes the elementary knowledge of astronomy contained in the previous one and builds on it to give accounts of black holes, quasars and active galaxies, the missing mass, novae and supernovae, Vulcan and transplutonian planets, and comets (a little surprisingly this, since comets are not as unusual as all that). Each chapter is written in a fair amount of detail, with the stages laid out logically by which astronomers came to their occasionally monstrous conclusions. The chapter on Eta Carinae is the most detailed in this respect, starting with its historical light curve, laying out just how Eta Carinae differs from other superficially similar kinds of stars,

discussing its infrared properties and Gratton's model of the star and concluding with its possible link with the Hubble-Sandage Variables.

The book would be enjoyed by an amateur astronomer who is ready to get to grips with some detail in astronomy. The accounts of the history of the subjects with which it deals make it a book which, say, graduate students would find it profitable to read, so that their thesis on the subjects in which they may be researching might then reference more than the last couple of years' *Astrophysical Journal*. The bibliography to each chapter gives enough references, typically to review articles of the kind that appear in *Nature*, for an easy entry into the literature dating before 1976 when the first edition of the book appeared. This, incidentally, is a translation of the second Italian edition (1979). I can pay the translation the highest compliment of all, that if it was not for a few footnotes accredited to the translators, one would scarcely notice that the book was translated.

Maffei's style is engagingly informal, although always scientific. The effects of close encounters of an astronaut with the tidal forces and event horizon of a black hole, for instance, are described with enough realism to make the image specific but not overplayed in the repellently sensational, even mystical, way which characterizes many journalistic accounts. On the contrary, this is a book which is written by an astronomer who knows the astronomical literature and has considerable skill in interpreting it at a popular level. □

Paul Murdin is an astronomer at the Royal Greenwich Observatory, Herstmonceux, UK.

The AI saga

Donald Michie

Machines Who Think. By P. McCorduck. Pp.375. (Freeman: Oxford, 1979.) £7.80.

THE task of the scientific referee is a well known contradiction. If he is himself a part of that particular action, then he cannot be impartial. If he is not, then how can he be technically competent in his task? The writing of scientific history is in similar case. It is therefore the more remarkable that Pamela McCorduck's gossipy saga on artificial intelligence, aspiring neither to impartiality nor to technical competence, has scored an undoubted overall success.

Her partiality is to the American part of the story, especially to Pittsburgh. Her saving gift is vitality, which carries her eagerly into previously unexplored catacombs of AI's pre-history. Using gold as the construction material the lame Greek god Hephaistos fashioned female attendants to help him walk. These robots not only walked, but also gave utterance from the promptings of inbuilt intelligence. A bronze android named Talos, Zeus' love-gift to Europa, patrolled the shores of Crete three times a day and hurled rocks at invaders. On catching any, he would heat himself up and fry the victim in a fiery embrace. Pandora, another female android from Hephaistos' shop, was developed under contract from Zeus to punish mankind for accepting Prometheus' gift of fire.

Joseph Golem was the artefact of the 16th century Loew, chief rabbi of Prague and friend of Johannes Kepler. The Golem's use was to spy on Gentiles. In reporting on his missions he was somewhat hampered by being mute.

Mary Shelley's tale is brought hauntingly to life, with Dr Frankenstein's creation driven half-mad with loneliness and his maker's evident physical repugnance for him. "Misery makes me a fiend!" says the 8-ft monster, and begs his master to fashion a soul-mate for him. Frankenstein, having promised this, abandons the task, with devastating effect on the creature's subsequent behaviour.

McCorduck's shrewdest move is to have gathered lengthy oral histories from numerous tape-recorded interviews for later interweaving with pre-existing records. It is startling to read Marvin Minsky's words of 1956:

The important result that would be looked for would be that the machine would tend to build up within itself an abstract model of the environment in which it is placed. If it were given a problem, it could first explore solutions within the internal abstract model of the environment and then attempt external experiments. Because of this preliminary internal study, these external experiments would appear to be rather clever, and the behaviour would have to be regarded as rather 'imaginative'.

The subsequent role of heuristically guided processes of search and inference performed on internally stored world-models has been central to AI. So too has the development of special forms of computer language in which to express what the machine knows about its world. It is therefore illuminating to read John McCarthy, saying at this same formative Dartmouth Conference that it was desirable "to attempt to construct an artificial language which a computer can be programmed to use on problems requiring conjecture and self-reference . . .".

Occasional flashes of this kind give welcome relief from the human-interest magazine style. But the latter also has its moments, not least when McCorduck permits herself to write directly from her own resources of wit and perception. On intellectual fashion:

No sooner do hemlines go down with

enormous fanfare than they go up again, the provinces growing dizzy with trying to keep pace . . . MIT thinks itself stylish, but outsiders have been known to call it faddish. Carnegie-Mellon, on the contrary, represents old-world craftsmanship, attending to detail and using the finest materials . . . But classic can be stodgy: if Queen Elizabeth of England bought artificial intelligence, she'd surely buy at Carnegie-Mellon.

For those not already members of the AI subculture, the parade of personalities, their doings and sayings, is liable to pall. Chapter after chapter is devoted to attacks by American polemicists of whom the general scientific world has never heard, aimed at targets whose names are scarcely better known, concerning philosophical issues about which few readers will excite themselves one way or the other. Yet during the same period a world-renowned British physical scientist published a concretely phrased indictment of robotics-

oriented AI activity in Britain, and this led to its almost total dismantling and dispersal. McCorduck passes over the consequential event with a brief mention: hardly excusable in a serious history.

But McCorduck's book is not offered as a serious history, any more than Aubrey's *Brief Lives* is a serious history of Oxford, or David Niven's *Bring on the Empty Horses* is a serious history of Hollywood. The reader should not come to Pamela McCorduck's whimsically titled book for instruction, or for any deep insight. But by stretching his limbs for a while in her brightly peopled landscape he can assuredly acquire, along with much enjoyment, a feel for the adventures, and the adventurers, of a scientific story at its beginning. □

Donald Michie is Professor of Machine Intelligence at the University of Edinburgh, UK.

Natural illustrations

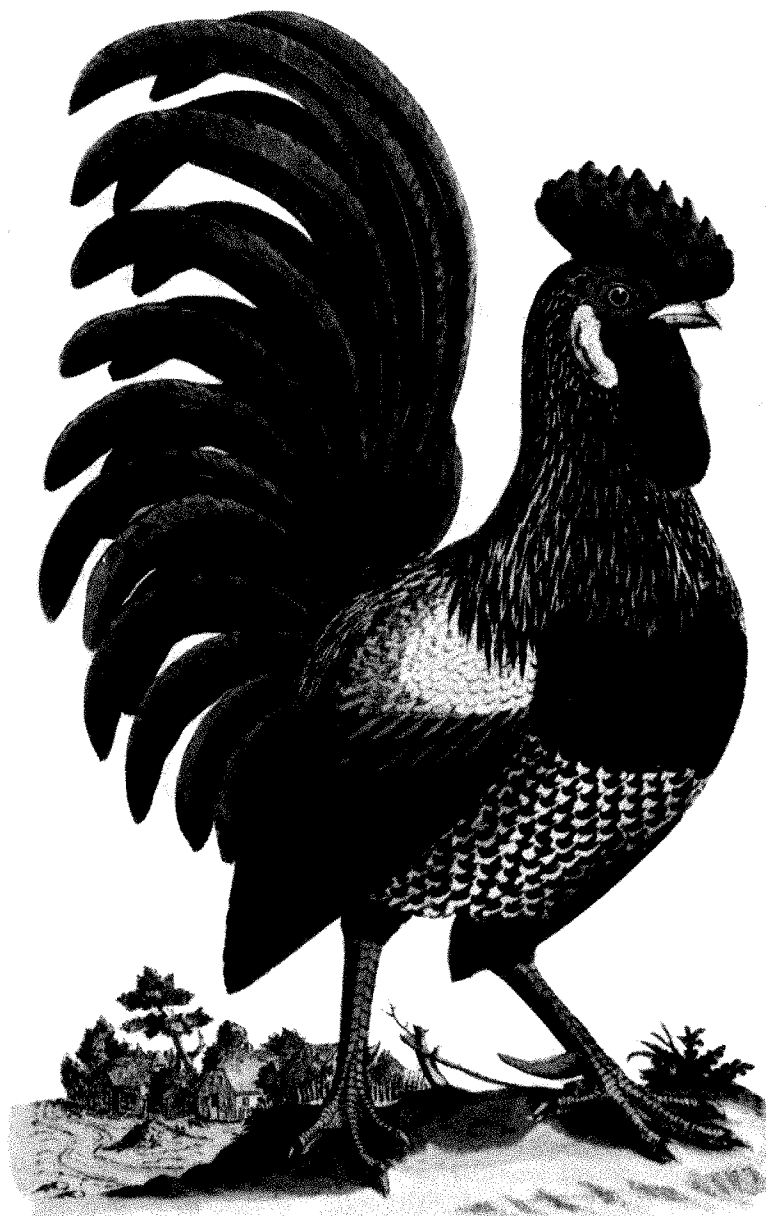
R.D. Meikle

Nature into Art. By H. Buchanan. Pp.220. (Weidenfeld and Nicolson: London, UK, 1979.) £15.

THE title neatly indicates the scope and object of this handsome volume. It is a concise survey of natural history books with fine, coloured illustrations, for the most part published between 1700 and the middle of the succeeding century. The accompanying text is concerned not so much with the scientific importance of the publications or the scientific exactitude of the plates as with their artistic merit and technical excellence.

To deal with such a vast subject within the compass of 220 pages means, of course, that even the finest illustrators are represented by only a very few examples of their work, while many of the less distinguished must be passed by, sometimes without as much as honourable mention. Selections seldom please everyone, but few are better qualified than Handasyde Buchanan, with forty years' experience of such splendid literature, to judge what must be included, and to decide — a more invidious task — what may be omitted. His choice is well balanced, without being too detached, just as his text is concise without becoming a bare catalogue of facts. The notes on printing techniques will be particularly welcome to those who have puzzled over *del.*, *dir.*, *sculp. imp.* and *pinx.*, or who have failed to grasp the distinctions between copper and stipple engravings, mezzotints and aquatints. An appendix of book prices

Plate from Johann Leonhard Frisch's *Vorstellung der Vögel in Deutschland*, Berlin 1733-63.



current in 1934 seems, at first sight, to be a catalogue of missed opportunities, until one recalls that, in the same year, a professional naturalist, lucky enough to find work, was considered affluent on a salary of £400 p.a.

Illustrations are essential to the botanist or zoologist, indeed it may be conceded that one good plate is worth a dozen paragraphs of descriptive text, however carefully penned. But it may be questioned if more than a very few of these "grand" works were intended for scientific study. Most were destined for the libraries of those enlightened sections of the gentry who, drawn to the study of plants and animals by the lucid codifications of Linnaeus, sought, in botany and zoology, a pastime less boisterous and bloody than the chase. With few exceptions the text which accompanies such glorious illustration is a sad let-down, even that of the monumental *Flora Graeca*, too often revealing an absence of accurate data, while the *Botanical Magazine* belied its name for a hundred years by serving up what often amounted to little more than the shadow of

a commentary.

The relative immobility of plants, and their clear, definite outlines, suited the processes of engraving; indeed, in the hands of skilled engravers, the very limitations of the technique were salutary, and, with the rise of lithography, plant illustration (always excepting the work of W.H. Fitch) too often degenerated into the blurred prettiness of *The Ladies' Flower Garden*. On the other hand, lithography might almost be said to have been made for the softer world of fur and feather. Set against the splendours of Lear and Gould, many earlier illustrations of mammals (and to a lesser degree, birds) have a quaint, almost comical look, even in books like Catesby's *Natural History of Carolina*, where the plant illustrations are perfectly acceptable. Ignorance of animal movement and anatomy was, no doubt, a factor also contributing to the comparatively late development of good animal illustration: the lifeless butterflies which Ehret and his contemporaries scattered so liberally over their botanical plates are, to my mind, an unwelcome adornment, detracting from,

rather than adding to, the beauty of the flowers.

So many aspects of *Nature into Art* are pleasing that it seems almost ungrateful to end on a sour note, but I doubt if anyone will commend the extraordinary system whereby groups of legends are referred backwards or forwards to invisible page numbers, a scheme which might have been designed to ensure maximum exasperation and minimum accuracy. Less irritating, because it may have been unavoidable, is the frequent spread of animal plates across facing pages, with a hinge down the middle, breaking the poor creature's spine, and sadly detracting from the artistic effect. These are, however, small criticisms of a book which will delight all those who value a tradition (fortunately still very much alive) which reconciles, in the happiest possible way, the several objectives of art and science. □

R.D. Meikle is a Principal Scientific Officer on the staff of the Herbarium, Royal Botanical Gardens, Kew, Richmond, UK.

Objectives for technology

Steven L. Del Sesto

The History and Philosophy of Technology. Edited by G. Bugliarello and D.B. Doner. Pp. 384. (University of Illinois Press: Urbana, Chicago and London, UK, 1979.) £12.25.

THIS book attempts to locate the problems of contemporary technology within the mainstream of philosophical and historical inquiry. It is the belated publication of a collection of papers first given at the International Symposium on the History and Philosophy of Technology, May 14-16, 1973, at the University of Illinois, Chicago Circle. The event was sponsored jointly by the Colleges of Engineering and Liberal Arts, attesting to the interdisciplinary character of the book and its principal subject matter. The book therefore reflects the essential beliefs of the members of the symposium: that technology plays an increasingly centralized role in our societies and our lives, and that we might study it systematically by way of the substantive and methodological approaches offered by history and philosophy.

To achieve this task, the book consists of some two dozen essays, structured around three sub-headings. The first, the history of technology, contains a number of useful papers clarifying the connections between history, society and technology. Among the papers in this section is an original piece by A.L. Donovan concerning the relationships between science and technological innovation, described by way of an

account of the close personal association between Joseph Black and James Watt in the invention of the separate condenser. There is also an insightful paper by N. Rosenberg discussing the complex interactions between technology, society, economy and values; he argues that we cannot predict the consequences of technological change without understanding the values of a society. And finally, some methodological suggestions for studying the relationships between science and technology are advanced by D.S.L. Cardwell and C.S. Smith. Smith posits that the ultimate understanding of invention and discovery depends more on the study of material objects than on the written records which historians generally use, while Cardwell argues that much of physical science as we know it was shaped by man's early industrial experiences, and not simply the other way around as many people believe.

The papers in the second section are grouped under the heading, the philosophy of technology. D. Wojick, for example, shows the utility of a Kuhnian model of scientific change to describe what he calls "technological revolutions" (nuclear power, food additives, and water resources management in the United States are his examples); while M. Bunge gets down to the serious job of characterizing the relationships between technology and the various branches of philosophy: epistemology, metaphysics, axiology, ethics and legal philosophy. In addition, there are papers concerning the relation between modern technology and political theory, as well as essays which trace the history of the philosophy of technology to its early origins. Many papers in this

section contribute detailed bibliographical materials that will be quite useful to specialists and philosophers, but are probably less interesting to the general reader.

The book concludes with a section on the future of technology which I found too brief and largely disappointing, perhaps because of the many new developments since the symposium was first held more than six years ago. I would like to have seen the important relationships between technology and social change explored more directly, and there could have been additional material regarding the anticipated affects of contemporary technological enterprises such as electronic message transfer, new biological techniques, computers, and so on. Furthermore, little attention was afforded either to the important issue of technology assessment, or to the areas of alternative technology and technology transfer. What we get are eloquently written but largely familiar statements like "technology subverts human values and impinges upon individual freedom", "we must understand technology to help provide for the continued existence of the human race", and the like. No-one denies the importance of such issues, but serious scholars of technology are apt to find the section on the future of technology less useful than the preceding ones; it is at best incomplete.

Beyond the content and organization of the individual essays, the book as a whole stresses at least two general themes, which seem to have been the central messages of the symposium. First, it is clear that the phenomenon of technology deserves continued, if not more systematic, study by scholars. Comprehensive studies and

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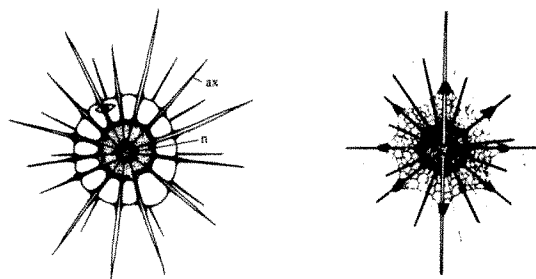
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The Uranium People

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programmes concerned with technology/society relationships should receive continued support. But the essential task, as M. Kranzberg notes in his introductory essay, is not simply study and reflection, but to embark on the difficult job of developing more precise conceptual, theoretical and methodological frameworks that allow the codification of diverse results from many fields of inquiry. Specifically, I would add that we need to work on higher level theories of social and technological change which can better explain how we got where we are, and where we might be headed in the future. This means, among other things, reaching basic conceptual agreement on key terms often used loosely and broadly — terms like “rationality”, “automation”, “efficiency”, “social change”, “science”, and the very concept of “technology” itself. Many of the essays in the book outline ways we may begin to tackle these important tasks.

Second, and I believe quite correctly, several papers put forth the theme that society must develop better moral and ethical perspectives on the control and improvement of technology, and that scholars of technology have a key role in the effort to produce better and more humane technologies in the years ahead. The job cannot be done simply by politicians, technocrats and administrators, but must be informed by the arts, humanities and the social and natural sciences as well. The task is truly interdisciplinary in character; and greater levels of improvement will not emerge without broadly based action which ranges far beyond the efforts of historians and philosophers working alone.

In sum, this compilation of essays will be useful to specialists as well as to general readers wishing to grasp the broad contours of the study of technology. It is well documented and mostly clearly written, although the lack of a subject index is annoying and hampers its value as a research tool; this, I believe, should be a major function of such a book. Nevertheless, I think anyone seeking an understanding of the full range of perspectives on technology and society will find time invested here worthwhile. The book's essays illustrate well how technology affects, both positively and adversely, social and political institutions, and how the human psyche — those ways in which we think, act, and relate to each other and the world — are influenced in directions that we are only beginning to foresee. It is to be hoped that such historical and philosophical materials can inform policy objectives, and help us decide on future technological choices as individuals and societies. □

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Understanding non-crystalline materials

D.C. Licciardello

Electronic Processes in Non-crystalline Materials. Second edition. By N.F. Mott and E.A. Davis. Pp.590. (Clarendon/Oxford University Press: Oxford, 1979.) £29.

THE second edition of this enormously successful book is faithful to the Mott and Davis style. The authors expand on their laudable attempt at unifying the vastly growing experimental literature in this field within the framework of some very general theoretical concepts. The book represents the single work which provides a focus to a field which, before Mott and Davis, had a divergent breadth.

The task is particularly monumental for at least two reasons. Firstly, the theoretical foundation on which the treatise is based is not well established. Indeed, the current literature is rife with new calculations which are at variance with some of the more fundamental ideas on which the exposition depends heavily, such as the minimum metallic conductivity. Secondly, the experiments being reported give results which still vary, to some extent, from laboratory to laboratory. This is partly due to the substandard quality of early experimental work, since only recently has enough attention been brought to the subject (largely through this book) to attract the interest of the world's best solid state physicists. Mostly, however, non-crystalline materials add a new dimension to the experimenter's problem in that materials must be characterized by method of preparation in addition to chemical makeup, e.g. one does not describe the generic properties of amorphous silicon.

The book describes the basic theory in the first four chapters. Care is taken, in traditional solid state format, to separate the non-interacting problem (Chapters 1–3) from the interacting one (Chapter 4). The basic theoretical problem, in the first case, concerns the behaviour of an electron in a random potential. The theory was first illuminated by P.W. Anderson in 1958, and Mott and Davis consider the Anderson mechanism for electron localization fundamental to the understanding of the properties of non-crystalline systems. The theory is discussed in some detail within the context of application to real systems. Thus, the exposition is much less formal than the original Anderson treatise and attempts are made to recast the concepts within a descriptive band theory. Herein lies the real value of this work for the solid state experimentalist.

The important problem of conduction in random media is discussed at length. The book demonstrates within fairly simple

analysis the absence of conductivity for Anderson localized electrons. The transition to conducting behaviour when the randomness is reduced or the electron density is increased is treated and Mott's notion of the minimum metallic conductivity is described.

The effect of correlation on transport and localization (Mott transition) persists and is expanded upon in the second edition. The term Fermi glass is used to denote a system of localized electrons in which localization may be due to randomness (Anderson) or electronic correlation (Mott). Mott and Davis discuss, as a class, those systems which undergo transitions from the Fermi glass state to metallic behaviour. To be sure, the two phenomena are distinguished, although the extent to which they are fundamentally different is again a current theoretical research topic. The discussion (Chapter 4) on metal-insulator transitions is unusual for the text in that the canonical examples of these effects are crystalline. Thus, Mott and Davis discuss doped semiconductors and narrow band transition metals.

The book, however, is mainly concerned with amorphous semiconductors. These are divided somewhat arbitrarily according to the local valence requirements of their constituents, Selenium and tellurium, however, are treated separately from the compound chalcogenide glasses. Mott and Davis make an invaluable contribution by extracting the most relevant details of the vast literature on these materials and by describing the basic electronic measurements in comparison. This kind of contribution defines the field and makes it particularly amenable to the newcomer.

Any treatise which ventures into a synthesis of a rapidly advancing subject runs the risk of being dated fairly quickly. The problem is even more serious when the authors engage, as is done here, in an explication of many measurements in terms of a general theoretical framework which, as pointed out above, is still controversial. For example, it is fair to say that at this writing, current renormalization group calculations give a conductivity at the Anderson transition with no minimum value. Much is made, in the second edition, of experiments on conduction in an inversion layer at the semiconductor/glass interface. The authors use this to support the notion of a universal minimum metallic conductivity in two dimensions. Again, there are recent calculations which suggest the absence of diffusion altogether in two dimensions. More recent experiments (not included in the book) tend to support this, although the mechanism for localization is still unclear.

The basic model presented for understanding non-crystalline semiconductors is a picture of an ideal structure into which the authors imbed a certain concentration of “defects”. The concept of a defect

within a random structure is difficult to define; however, the idea is that the electronic properties of the ideal structure would be similar to that of the corresponding crystal (if one exists). Thus, the defect, in this sense, is important in determining the specific electronic properties of the amorphous semiconductor. This is a rather extreme point of view for the structure of glass, in my opinion, yet the authors are able to cast much of the data within this framework. It is fair to point out, however, that models do exist which minimize the effect of local

structural inhomogeneities but do enjoy similar success in explaining much of the data. These theories invoke a strong lattice interaction and, indeed, Mott and Davis discuss the simple negative-U model first introduced by Anderson but narrowly interpreted here. Thus, the basic electronic structure for glassy semiconductors is not universally agreed.

The reviewer with the broad view may dismiss much of the current controversy as detail and indeed it is somewhat inconsequential when the contribution is taken as a whole. It is certainly true that some of the

analysis given in this book will be finally shown to be incorrect. This is the risk the authors boldly took in attempting to characterize and define a new branch of solid state physics. It is fitting that the Nobel Prize in physics was awarded to Sir Nevill Mott for contributions to this field, many of which are included in this book. □

D.C. Licciardello is an Assistant Professor of Physics at Princeton University, Princeton, New Jersey.

Heavy stone circles

R.J.C. Atkinson

Rings of Stone: The Prehistoric Stone Circles of Britain and Ireland. By Aubrey Burl, with photographs by Edward Piper. Pp.280. (Frances Lincoln/Weidenfeld and Nicolson: London, UK, 1979.) £9.95.

AUBREY BURL'S position as the leading authority on stone circles was established by the publication of his *Stone Circles of the British Isles* (Yale University Press, 1976), a work for scholars which is not likely to be superseded within the present century. *Rings of Stone* is a companion volume for the layman, extensively and admirably illustrated, but unencumbered by the *apparatus criticus* of scholarship. There is a glossary, a reading-list confined to books and monographs, and an index. The reader who is stimulated to seek primary sources must consult the bibliography and gazetteer of his earlier book.

Apart from its weight (1.2 kg) and its 27 colour plates on art paper, which are at the mercy of the fine soft rain which falls so

frequently on stone circles, this is an excellent vade-mecum for the holiday traveller with a purpose, whether it be specifically archaeological or aesthetic. The amateur prehistorian will find it useful in guiding him to the best-preserved and most easily visited sites. The traveller bent, in the Romantic tradition, upon viewing these rugged and enigmatic works of man against their landscape background will be well served also, provided that he remembers that the rocky and treeless terrains which now surround many stone circles are themselves the product of man (and indeed of the builders of the circles themselves), and not of nature alone.

Readers with either purpose will be guided in their travels by the latter two-thirds of this book, which describes and illustrates fifty stone circles in Britain and Ireland which are well preserved and easily visited. For each region there is a map showing the sites and the main roads. For each site there is a map reference and a plan and photographs, as well as a textual description which will delight the reader by its aptness and sensitivity, either from his prior knowledge of the monument or after his first visit. It is a pity, however, that there are no figured scales for the plans, which vary widely in their ratio of

reproduction; but the principal dimensions are given in the headings.

The photographs, of which there are 106 in black-and-white as well as those in colour, are mainly the work of Edward Piper, who has inherited much of the genius of his father, the artist John Piper. Apart from one or two of the colour plates, in which the printers have achieved an overall hue which is displeasingly chlorotic, this is the finest set of pictures of prehistoric antiquities which has ever been assembled between one pair of covers. It is a picture-book in its own right, quite apart from the accompanying text, and it will stimulate the reader to see more of these sites than the photographs can show. For those who are unable, or unwilling, to venture far from the slippers comfort of their own firesides, the combination of visual and verbal description conveys very well the variety of size and character of stone circles, and readily explains their appeal to the antiquaries and topographers of the Romantic Age, which is still happily not dead.

This guide for the visitor is preceded by ten short chapters, in which Burl summarizes his unrivalled knowledge of the stone circles of the British Isles and gives his own views about their purpose and date. He begins with one of the principal regional groups, which is also morphologically and archaeologically the most coherent: the Recumbent Stone Circles of north-eastern Scotland. Here ten or eleven stones, rising in height towards a pair flanking a recumbent slab lying on edge and often with a top surface accurately horizontal, surround a low annular cairn of small boulders with a small central open space, in which excavation has frequently revealed signs of burning and small deposits of cremated human bone, accompanied significantly often by fragments of quartz.

The paucity of the human remains leads Burl to conclude, and doubtless rightly, that these were not cemeteries, but places of worship for a single extended family, sanctified at their inception by a token burial or burials, perhaps as the sequel to deliberate sacrifice. He notes, however, that although a single family could have



Edward Piper.

raised all the upright stones, the transport of the recumbent stone (typically of 20 tonnes or more) required the combined efforts of the able-bodied members (and perhaps the males only) of a number of families. This provides a tantalizing glimpse of community action, and perhaps of a clan organization, in the late third and early second millennium BC; but its details are beyond the limits of valid inference from the evidence.

Burl also shows, in more detail than in his earlier book, that the position of almost all the recumbent stones lies within the azimuth range of the moon at its minimum declination; and he therefore posits a symbolic link between the moon and the dead who sanctified these sites.

In the remaining hundreds of stone circles elsewhere there is little to be seen of any recurrent pattern, save that they are of stone and approximately circular. Some but not all of the few which have been comprehensively excavated have produced burials, but again of a token or dedicatory kind, or as manifestly later additions, in the Early Bronze Age, to monuments built centuries before. They vary greatly in size,

from the huge ring at Avebury to tiny circles which would very nearly fit into the study in which I write this review; and in date they seem to cover a period of about two thousand years, starting around 3,500 BC. Within such wide ranges it is probably futile, as Burl implicitly recognizes, to seek any simple or universal explanation. It is better to admit, however reluctantly, that there are many interesting questions about the prehistoric past which are *per se* unanswerable, simply because there are no documents to give a clue to motives. Motive, except in the trivial sense of intending to do what was done, cannot be inferred from inanimate objects and structures. In answer to the question 'Why?' the evidence is dumb.

This otherwise excellent book dismisses too forthrightly the putative evidence from stone circles for the early development of scientific concepts. For Burl, the megalithic yard is "a chimaera, a grotesque statistical misconception". It is indeed a product of statistical inference; but two independent treatments of the data by different means have agreed, at the least, that further investigation is necessary

(*Trans. R. Soc. Lond. A276*, 1974; *J. R. Stat. Soc. A139*, 1976).

Burl likewise implicitly rejects Thom's hypotheses about how the non-circular rings (flattened circles, eggs, compound rings) were set out, on the grounds that alternative constructions, using only pegs and a loop of measuring cord, yield an equally good fit to the stones surveyed. He may be right; but he ignores or evades the point that even if these shapes, manifestly deliberate and not accidental, are no more than the fossilized remains of games with pegs and string, they none the less constitute the foundations, gropingly laid maybe, of pure mathematics.

The astronomical aspects of stone circles can be assessed only statistically, using the largest samples available, because putatively significant alignments can obviously occur by chance. Independent checks of the claims of Thom and his followers are only now in progress. Their outcome should not be prematurely judged, in advance of publication. □

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Electronic theory of cancer

M. M. Coombs

Submolecular Biology and Cancer. Ciba Foundation Symposium, No. 67. Pp. 349. (Excerpta Medica: Amsterdam, 1979.) \$42.50, Dfl. 87.

THIS book reports the proceedings of the 67th Ciba Foundation symposium, held in honour of Albert Szent-Györgyi on the occasion of his 85th birthday in 1978.

In an introduction by the Chairman (R. J. P. Williams) it is noted that although "submolecular" refers to systems at a lower level than whole molecules, in the present context it is largely confined to electrons. Szent-Györgyi next outlines his views on "the living state and cancer", and the possible relationships between submolecular biology and cancer are enumerated. The greater part of the book is devoted to fifteen lectures, each with its own discussion, and ends with a general consideration of Szent-Györgyi's hypothesis.

The sharp distinction between living and inanimate matter leads Szent-Györgyi to conclude that a specific physical state underlies life. The subtle reactivity of living systems is thought of as an inherent property of the constituent proteins, brought about by removal of single electrons from their otherwise fully occupied orbitals. Positive electron holes

thus produced lead to electrical conductivity, with the ability of the desaturated proteins to carry information in the form of electrical signals. It is proposed that "the living state is the electronically desaturated state of molecules, and the degree of development and differentiation is a function of the degree of electronic desaturation". When life began on Earth the atmosphere lacked molecular oxygen; electronic desaturation of proteins was accomplished by simple aldehydes, such as methylglyoxal ($\text{CH}_3\text{CO}\cdot\text{CHO}$), which are good electron acceptors. This led to a low degree of development, termed the α -state. Later the advent of atmospheric oxygen, a much better electron acceptor, led to the highly developed β -state existing today. Here a related molecule, ascorbic acid (vitamin C), acts essentially as a catalyst for the electronic desaturation of proteins by oxygen. Cancer is thought of as a reversion to the α -state.

It is obvious from the studies described in the subsequent chapters that little evidence can be found for electrical conductivity in proteins treated with electron acceptors, although here the virtual impossibility of making measurements on proteins in their native state may obscure true results. Nor is the anti-cancer activity of certain aldehydes apparently mediated in the way suggested by the hypothesis; moreover, it now seems very doubtful whether vitamin C is useful in the clinical treatment of the disease. Furthermore, experiment does not support the idea that there are differences in free radical levels between normal and tumour



Albert Szent-Györgyi.

Courtesy of Owen & Moroney, London.

cells. However, R. Damadian, in developing the technique of whole-body nuclear magnetic resonance scanning, can distinguish normal from cancer tissue by the prolonged relaxation of protons in the latter. This indicates increased disorder in the structure of water in tumours, but just how this ties in with Szent-Györgyi's theory is unclear. To summarize, it is disappointing that this novel hypothesis has so far failed to find solid experimental confirmation.

The book is well produced and free from errors; its cost does not seem unreasonable in this age of unreasonable prices. □

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Fludd's philosophy

P. M. Rattansi

Robert Fludd and His Philosophicall Key. Introduction by Allen G. Debus. Pp.156. (Neale Watson: New York, 1979.) \$40, £20.

IN 1620 Francis Bacon published his *Novum Organum*, with a dedication to James I, urging him to support his enterprise, "that so at length, after the lapse of so many ages, philosophy and the sciences may no longer float in air, but rest on the solid foundation of experience of every kind . . .". At about the same time, the physician Robert Fludd had completed a *Philosophicall Key* "to unlock and open the meanings of that Macrocosmicall and Microcosmicall Philosophy". He, too, dedicated it to James I, hoping the King's patronage would shield him "from the Harpys talents and envious endeavours of this World".

Fludd agreed with Bacon that Aristotelian science was to be replaced by one based on "ocular demonstration" and dedicated to the relief of human misery. Like him, he praised atomism, but was more inclined to explain nature's operations in terms of a 'spirit' active in all things. But Fludd believed in a 'Christian' philosophy of nature, the principles of which would be drawn from the account of creation in the text of Genesis. Bacon condemned the "unwholesome mixture of things human and divine" since it gave rise to "not only a fantastic philosophy but also an heretical religion". Fludd associated the greatest secret in nature, the principle of life, with light and the breath of the Lord, while Bacon denounced such notions as "superstitious".

His major works had already appeared in Latin, and had involved Fludd in controversies with Kepler, Mersenne and Gassendi. The *Key* was written in English to silence critics at home. Fludd thought his "experimental demonstrations" would convince readers of the truth of his ideas. Relying on an analogy between the Sun in the Universe, the heart in the human body, gold in the metalline and wheat in the vegetable kingdoms, Fludd chose to concentrate on experiments on wheat. After all, had it not been compared to the Second Person of the Trinity, and was it not Christ's referring to bread as his body which had set the Christian world "in combustion"?

The planting of the seed in the ground and the generation of wheat had exemplified the pattern of death and resurrection to the Christian alchemist. Fludd pointed out that as the plant grew, the quintessence in it rose to the stalk and was concentrated in the wheat grain. By a sympathetic magnetism, the most spiritual part of the plant would then draw down

more and more of the aerial spirit. This process could be emulated by the chemist, and Fludd believed he had succeeded in obtaining the universal spirit in the form of a "white crystalline" substance.

Fludd's claims may seem extravagant to us, his reasoning contorted and his "ocular demonstrations" quite unconvincing. It is disconcerting, therefore, to recognize in his work themes which recent historical research has unearthed in the alchemical studies which occupied a great deal of Isaac Newton's most creative period: the true interpretation of the alchemical foundation text, the 'Emerald Tablet'; the 'magnet' to draw down the aerial quintessence; and the involvement of light in generation in the three kingdoms of

nature. He represents a style of thought which cannot be neglected if we are fully to comprehend those whom we recognize as the pioneers of modern science.

The *Key* has been published for the first time from the manuscript at Trinity College, Cambridge, with an Introduction by Allen G. Debus. The text itself occupies 93 pages and has been supplemented with an extensive account of the life and work of Fludd. The work, written in a rhapsodic style which occasionally breaks into verse, makes Fludd's ideas a little more accessible to the modern reader. □

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Recombination in phage and fungi

H.L.K. Whitehouse

Genetic Recombination: Thinking about it in Phage and Fungi. By F. W. Stahl. Pp.333. (Freeman: San Francisco and Oxford, 1979.) £12.90.

THE publication of a book on genetic recombination by one of the leading workers in the field is an important event for geneticists. Recombination was discovered in the early years of this century and became a central issue in genetics with the realization that exchange of segments between homologous chromosomes was involved, in addition to the random orientation of the paternal and maternal members of each chromosome pair on the spindle at the first meiotic division. Recombination took on a new dimension about 30 years ago with the discovery of its occurrence in prokaryotes, and with the recognition that both in prokaryotes and eukaryotes understanding the process of exchange was likely to require detailed knowledge of the biochemistry of DNA. Since that time it has been essential, if a general view of the subject was to be obtained, that results derived from prokaryotes and eukaryotes should be integrated. To achieve such integration is difficult, as the life-cycles, and therefore the experimental techniques and kind of information obtained, are so different: the prokaryote- and the eukaryote-worker speak such different languages that they may barely understand one another. It is greatly to Stahl's credit that he has attempted in this book to bring about a synthesis of results from bacteriophages and from fungi, as these are the organisms that have contributed most from each kingdom. The phages discussed are lambda and T4 of *Escherichia coli*.

The first two chapters give the basic information about the exchange process obtained from progeny chosen on a random basis, in fungi and phages, respectively; and the next two chapters give the fuller picture when both products of an exchange are recovered — that is, tetrad analysis and single burst analysis. The implications of the duplex structure of DNA are introduced in Chapter 5. Then follows an important chapter on the surprising results that have been obtained, both in phages and fungi, from investigations with very closely linked mutants. Together with an appendix giving the algebraic basis of the concepts introduced, this chapter constitutes one quarter of the entire book. The following two are concerned with conversion and its association with crossing-over; and, after a consideration of recombination chemistry, the book concludes with lengthy discussions of current hypotheses. There is no account of transposons or phage Mu.

The lay-out of the chapters, although logical, does not necessarily make the subject matter easy to comprehend, and certainly leads to considerable repetition. Thus, having learnt in the first chapter that crossing-over takes place between homologous chromosomes, one has in a later chapter to replace this with the notion that the exchanges occur between daughter chromatids rather than whole chromosomes. Again, in discussing aberrant asci, one of the five basic kinds is treated in a different chapter from the others, because it does not have unequal numbers of the two parental genotypes and therefore does not qualify for consideration in a chapter on conversion. Yet all the types of aberrant segregation are associated with crossing-over and it is arguable that the simplest approach is to treat them all together.

Some of the difficulty for students in understanding what is known about genetic recombination derives from complexities inseparable from the organisms investigated. Thus, in lambda not only are there two recombination

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systems specific to it, but the host recombination pathways also act on the phage. Furthermore, the method by which lambda packages its DNA in the head of the virus probably leads to a bias in the recombination events shown by the progeny. With T4 the complexity of the experimental results arises from the extraordinary genetic system that prevails in this phage, which has linear genomes that are circularly permuted with respect to one another. Although a concise account is given of the relevant results, it would be an advantage to students if they already had some knowledge of recombination studies with *E. coli* and these phages.

The book is addressed to graduate students and final-year undergraduates, but I fear that many will find it difficult reading. The problems at the end of each chapter will no doubt help towards a better understanding, though some of these will certainly present a severe challenge for students. Fortunately, the solutions are given later in the book. The clarity of thought that the book constantly demands is helped by a profusion of unnumbered small diagrams which form an integral part of the text. In some of the diagrams the differences in line thickness that indicate the parentage are easily overlooked, and there are no legends to draw one's attention to them. The author's entertaining, often colloquial, style helps to enliven the book.

Errors are few, but Figures 8-1 and 8-2 have been transposed and an incorrect reference is given in this chapter for data on

Sordaria asci with 5:3 ratios for spore colour. In the previous chapter Rossignol and Haedens' work is discussed without reference to them. There is a useful glossary but Stahl's new term, 'splatch', is not included though freely used in the text. It is surprising in a book on genetic recombination to find no reference to the important contributions to the subject by Benbow *et al.*, Ephrussi-Taylor *et al.*, Spatz and Trautner or Tiraby *et al.*, even though their work relates to organisms other than those with which the book is primarily concerned.

My chief criticism of Stahl's book is that it does not give a balanced view of the current state of knowledge of recombination in eukaryotes. Hypotheses involving fixed pairing segments, such as his so-called "sex-circle" model, are discussed without drawing adequate attention to the conflict they present with a considerable body of data. He lists eleven features of the recombination data for *Saccharomyces cerevisiae* and implies that these results are so different from those found with other fungi as to demand a quite different explanation. But most of the results with yeast have also been found in other fungi; and to me the similarities are much more impressive than the differences. He supports the idea of break-and-copy by reference to work on phage $\phi 1$ without acknowledging that the authors subsequently abandoned this hypothesis for a more conventional one depending on heteroduplex DNA. And why suppose that

in fungi two crossovers (reciprocal exchanges) may occur in very close proximity in the same two chromatids when there is no evidence for this? Stahl repeatedly raises the subject of interference between crossovers, even to the final sentence of the text, where he says "... we would all feel better if a good molecular explanation for the facts of interference were neatly wedded to some of those models", referring to those he has been discussing. But he seems to overlook the very great distances, in molecular terms, over which interference often acts, and the lack of it across the centromere. It seems likely that interference is a phenomenon superimposed on the basic crossover mechanism rather than a primary feature of it. When discussing Holliday's recombination model in Chapter 8, Stahl argues that interference should lead to an excess of recombinant over parental flanking marker genotypes in association with conversion, but Holliday himself (*Phil. Trans. R. Soc. B277*, 367; 1977) draws the opposite conclusion.

Despite these criticisms, Dr Stahl is to be congratulated for producing such a challenging and thought-provoking, if controversial, work. It should draw attention to the many unsolved problems in this field and provide a stimulus for further research. □

H.L.K. Whitehouse is Reader in Genetic Recombination in the Department of Botany, University of Cambridge, UK.

Illustrated black holes

R.L. Znajek

Black Holes. By W. Sullivan. Pp.303. (Anchor Press/Doubleday: Garden City, New York, 1979.) \$17.95.

SULLIVAN'S *Black Holes* begins with a bang. Literally. The first chapter is about the Tunguska event — the great Siberian explosion of 1908. This is tenuously linked to the title by a theory that the explosion was caused by a small black hole falling through the atmosphere (Jackson and Ryan, *Nature* 245, 88-89; 1973). Like all good theories it made a prediction: the hole passed through the Earth and emerged over the North Atlantic Ocean, causing a similar explosion as it left. This should have caused atmospheric vibrations comparable to those detected from the original event. It turned out that no such vibrations were detected from the North Atlantic (Beasley and Tinsley, *Nature* 250, 555-556; 1974), and so the theory now seems untenable. There is of course no shortage of other ideas, ranging from the exotic (an

exploding nuclear-powered spacecraft) to the quite plausible (a small comet or meteorite). Sullivan tells us about all of them, and this gets him into digressions about anti-matter and carbon-14 dating. We are treated to maps, eyewitness reports and even photographs of a man who was blown off his porch by the blast. It is mostly quite interesting. Like the rest of the book, it has little to do with black holes. But the title presumably sells.

Black Holes is in fact an account of some of the more dramatic goings-on in modern astronomy. It deals with white dwarfs, pulsars, X-ray sources, quasars and cosmology, as well as with things that might have exploded over central Siberia. Sullivan, being Science Editor of the *New York Times*, writes in an appropriately journalistic 'who-did-what' manner. He emphasizes observations and is short on theory. The trouble is that black holes are inherently theoretical objects. They cannot, by definition, be seen or photographed. Events happening near a black hole can be observed, and the hole's presence deduced theoretically. A book about black holes should certainly explain why many astronomers think they exist, and this one does. It should also make a serious attempt to explain what they are,

and this means more than just stating that gravity becomes so strong that not even light can escape.

I am not asking for a layman's guide to tensor calculus and the Einstein equations. I am suggesting that one can give a precise but non-mathematical account of relevant aspects of special and general relativity, such as light cones and frames of reference. By showing how light cones are tilted at the surface of a black hole, one can demonstrate why the surface is an 'event horizon' and why leaving it is precisely equivalent to travelling faster than light. (A spaceship that can travel faster than light can get out of a black hole. Scientists depicted in the recent film *The Black Hole* seem surprisingly ignorant of this fact.) Laymen may not understand mathematics, but most of them can read maps, and so any popular account of black holes should include plenty of space-time diagrams, which are just maps showing the location of events in space and time. The best example of what I have in mind is Kaufmann's book *The Cosmic Frontiers of General Relativity* (Little, Brown: Boston, 1977), which might almost be accused of being over-illustrated. Sullivan's book is crammed with photographs of astronomers, telescopes, rockets and galaxies, but I cannot

find a single space-time diagram in it.

Having made my complaints, I must confess I quite liked the book. All that is wrong with it is the market it is aimed at. It is basically an anecdotal history, and as such is more likely to interest astronomers than the general public. It provides a personal dimension which is normally absent in original papers and review articles. Sullivan records the comments of scientists in the process of discovering, debating or just feeling confused. The result is somewhat chaotic, but that is what

science is often like. Sullivan himself describes his book as "an ongoing scientific mystery story". He provides an excellent set of "Selected References", the main merit of which is that they are not selected. Most of them are very inappropriate for laymen, but they are precisely what is required by astronomers wishing to get to the science behind the anecdotes. The book would be improved by the removal of some of the weaker attempts at popularization, such as the summing-up of diffraction as "light plus

light equals darkness". There are a few inaccuracies, like the statement that in an accretion disk "gas would spiral in more rapidly were it not for friction". (Gas would not spiral in at all were it not for friction.) I can nevertheless recommend this book for the history section of any astronomical library. I would recommend Kaufmann's book to any layman who wants to know about black holes. □

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Thames revival

R.J. Wootton

The Tidal Thames. By Alwyne Wheeler. Pp.228. (Routledge and Kegan Paul: London and Boston, 1979.) £8.95.

THE tidal Thames runs from Teddington Lock, west of London, eastwards to the North Sea. Bisecting the capital city, this length of the river has streamed through British history, providing a means of transport for men and their commerce. Until the nineteenth century, it also supported flourishing fisheries which supplied London with such esteemed food fish as salmon, smelts, eels and whitebait. But thereafter a combination of events destroyed these fisheries and left a long stretch of the tidal river apparently lifeless. Only in the past two decades has this degradation of the river been reversed, and now fish can once again be found throughout the tidal Thames. Alwyne Wheeler, an ichthyologist at the British Museum (Natural History), has played an important role in recording the return of fish to the Thames. His book tells of the death and revival of the river as a habitat fit for fishes.



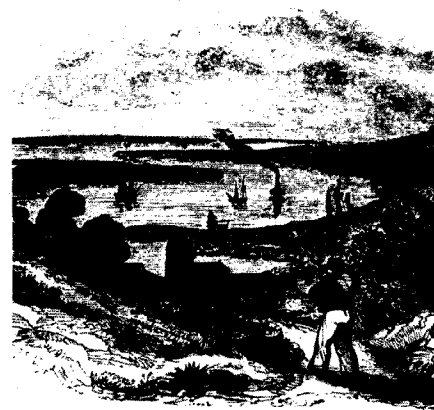
In the first half of the book, Wheeler describes the decline in quality of the river and its effects on the fisheries. Industrialization and population growth were the major factors. Pound locks were constructed to improve navigation on the river, but these hindered the movements of migratory fish and made their capture easier. Gas works and other industries poured their toxic by-products into the river. The primitive methods for the disposal of human excrement, cess pits with the night soil spread on agricultural land, became inadequate so that disposal

through sewers was introduced, a development encouraged by recurrent outbreaks of cholera. At first the sewers discharged directly into the river in London, but this led to such gross pollution that on an infamous occasion in 1858, "the year of the great stink", committee meetings in the Houses of Parliament were suspended because of the stench. A great Victorian engineering scheme was initiated, and in a few years 84 miles of sewers had been built which carried London's wastes eastwards to empty into the river downstream of central London. Sadly, the site for the outfalls was miscalculated, and the sewage was trapped in the water mass by tidal movements. This area of the river, receiving most of London's excrement, became so polluted it was claimed that suicides died of poisoning before they could drown themselves. If the outfalls had been a few miles downstream it is possible that the Thames would never have become lifeless. Although the situation had improved by the beginning of the twentieth century, two world wars, and the continued growth of population in the London area without adequate provision for sewage treatment, meant that by the 1950s there were no established fish populations in the Thames over a distance of 69 km. Most of this stretch had extremely low dissolved oxygen concentrations, the consequence of the massive organic pollution.

This intolerable state was recognized and major improvements in sewage treatment started. The effect of these improvements on the fish fauna was monitored in an original way. Wheeler arranged for the collection of fish caught on the filter screens of electricity generating stations which extracted cooling water from the Thames. These collections yielded a clear picture of the recovery of the fish fauna, as both the number of species and the number of individuals increased. This picture of a cleaner river was emphasized dramatically when salmon were recorded in the Thames for the first time in 140 years.

The second half of the book is a detailed description of the results of Wheeler's and other collections made since 1967, in which 72 species were recorded. This section makes difficult reading. Much of the

quantitative data could have been relegated to an appendix for, as Wheeler himself notes, the method of collection provided a qualitative rather than quantitative picture of the recovery of the fish fauna. Yet some of the detail is inadequate: for some species inadequate morphological detail is given; and authorities are not given for the specific names while some common names are used inconsistently.



Finally, Wheeler notes that a cleaner river is more sensitive to environmental disturbances which would have been insignificant when the river was lifeless. Potential hazards include the effects of large power stations on water temperature and hence dissolved oxygen, the discharge of raw sewage during storm overflow, and the effects of flood prevention schemes. Although it is unlikely that commercial fishing will again flourish in the tidal Thames, the aesthetic and recreational benefits provided by established fish populations are incalculable. Wheeler's book illustrates how effective public spending, which is now under attack, can be in a civilized, industrialized state.

Although there are a few typographical errors and mistakes of chronology, the book is reasonably priced. Many of the illustrations are taken from Yarrell's *British Fishes* (1859), which links Wheeler's book with that of a naturalist familiar with the Thames before it died of pollution. □

R.J. Wootton is Lecturer in Zoology at the University College of Wales, Aberystwyth, UK.

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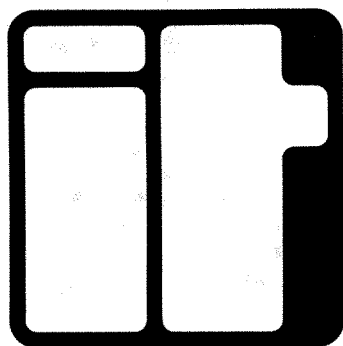
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Surprise!

J.S. Bell

Surprises in Theoretical Physics. By R. Peierls. Pp.166. (Princeton University Press: Princeton, 1979.) Hardback \$19, £8.40; paperback \$5, £2.10.

THIS is a fascinating book. About thirty situations are presented, scattered widely over theoretical physics. The surprise is sometimes that the obvious answer is wrong, sometimes that the devastating criticism of the obvious answer is wrong, sometimes that a very great man has gone wrong, and so on. Some of the surprises are pleasant, as with the validity of the nuclear shell model, or with the simplicity of Landau's diamagnetism. Some of the surprises are unpleasant, as with the non-existence of the Bogolyubov expansion for the diffusion coefficient, or even with the difficulty of estimating the adequacy of the JWKB method in barrier penetration. Some of the distinguished men who slipped up were very distinguished indeed. Debye was quite wrong about thermal conductivity in non-metals, and the "reasonable agreement with experiment fortuitous". Heisenberg was wrong about the resolving power of his γ -ray microscope, and was corrected by Bohr. Einstein forgot his own gravitational redshift in an attack on the time-energy uncertainty relation, and he also was corrected by Bohr. But Peierls himself remains surprised that Bohm and Aharonov have successfully attacked a version of this "fourth uncertainty relation". He conjectures that the interaction they invoke "for some reason cannot be permitted in quantum mechanics".

Sometimes the important reference is to early work of Peierls himself. It is the 'Umklapp' process of his 1929 thesis which resolves the difficulty with Debye's thermal conductivity. Sometimes the surprise has been generated by colleagues in his own department. But the choice is catholic, and indeed the two longest sections in the book are concerned with two perennial surprises — irreversibility in statistical mechanics, and the problem of interpreting quantum mechanics.

It seems to me that the non-technical account of macroscopic irreversibility, contrasted with microscopic reversibility, brings out admirably the essential point often concealed in lengthier and more mathematical accounts. That is to say that the situation is intelligible when we suppose boundary conditions to be imposed in the past, rather than the future, and with no great care — or at least without the fantastic and conspiratorial care that could have ensured the exceptional decrease of entropy rather than the normal increase.

As regards the problems of quantum mechanics, Peierls begins with an account

of what went wrong with the celebrated von Neumann theorem on the impossibility of deterministic hidden variables. He continues with a nicely ironical account of subsequent work centred on the notion of 'locality'. Then he takes up the infamous 'reduction of the wave packet'. To my dismay he regards it as "clear that the significance of the state function is to represent our state of knowledge of the system". But he goes on to ask "whose knowledge . . .", and is carried down into the depths. He finally conjectures that the Schrödinger equation does not apply to conscious organisms and (if I interpret correctly) that it is in the presence of such organisms that linearity fails and wave function collapse occurs. It seems to me that the reduction is then dynamical rather than actuarial. It is not at all to be equated with the mere adjustment of odds appropriate when a candidate for life insurance is seen to be over one hundred.

The bulk of the book is occupied with quantum and statistical mechanics. But finally there is one relativity problem. It is the old question of radiation — or non-radiation — from a uniformly accelerated charge. Here (surprise) there is something quite new, from unpublished work of Boulware. He has worked out carefully (for the first time, it seems) how it looks in the accelerated system in which the charge is at rest. I would have liked to have seen my favourite relativity surprise included in this book. I will yield here to the temptation to describe it (A. Evett, *Am. J. Phys.* **40**, 1170; 1972). Two identical spaceships,

identically programmed, are initially at rest in some inertial system S, one of them 100 metres behind the other. At a given moment (in S) both motors start and off they go. With identical acceleration programmes they remain, of course, always 100 metres displaced from one another (in S). The ships are initially connected by a fragile (but incombustible!) thread. This thread would like to Fitzgerald — contract as the ships speed up, but as they do not come closer (in S) the thread cannot contract — so it breaks. Or does it? My experiences with this puzzle have convinced me that most relativity courses seriously damage the minds of most students.

There are no surprises here from elementary particle theory. Is that, then, only a dull plodding sort of subject? I think not. For example, there was the big surprise of a renormalizable theory of weak interactions. And I think also of the very beautiful surprise of the 't Hooft-Poynakoff magnetic monopole. And surely there are many others.

But let us be grateful for what we do find in this excellent book. One of the nicest surprises in it is the elegant simplicity with which nearly all of these topics are presented. The essays are mostly accessible to undergraduates with a first course in quantum mechanics, and to graduates who have not forgotten. □

J.S. Bell is a Physicist working at CERN, Geneva, Switzerland.

Memorial to the Ghetto

John Rivers

Hunger Disease. Edited by Myron Winick. Pp.261. (Wiley: New York and Chichester, UK, 1979.) \$19.80, £10.60.

THIS is an important book. It was conceived of early in 1942 by Dr Israel Milejowski who, as chief of public health in the Warsaw Ghetto, was sure that death from starvation and disease awaited its inhabitants. Certain of this fate, and powerless to prevent it, he recruited 27 colleagues and persuaded them to undertake a scientific study of the effects of hunger that they were observing in the Ghetto. Such a study, he hoped, would be, at least, a memorial to the dead.

They had just five months to build that memorial. On 22 July 1942 the German authorities began to liquidate the Ghetto and its inhabitants. Milejowski records that their already meagre hospital and laboratory facilities were destroyed, and, with mass deportations occurring around them, the scientists wrote up their work

and prepared the manuscript that was smuggled out of the Ghetto and hidden.

It was a remarkable feat, but ironically it almost seemed as if their efforts were in vain. Though the manuscript survived the war, only limited editions were published in Polish and French, and though the title *Maladie de Famine* was often referred to in the nutrition literature, the document itself was largely unread by nutritionists.

In rescuing it from obscurity, and producing not only an English translation, but a detailed appreciation of the modern significance of the work, Professor Winick has ensured that the memorial Milejowski intended has at last been built.

But the book must not be viewed as a purely historical document, and Professor Winick has wisely treated it as a work of current scientific merit. Of course it is a strange scientific document, exploring theories and using techniques of 40 years ago. But outmoded or not it is still of current interest, because, with the exception of Keys' *Biology of Human Starvation* (University of Minnesota Press, 1950), it is the only study in the field. Though by its brevity it is less authoritative than Keys' work, it ranks alongside it as a scientific document because it deals with some different aspects of starvation, and



Severe marasmus and death in the Warsaw Ghetto.

because, unlike Keys' experimental study, it deals with people starving to death.

I am sure that many of the results the authors obtained will still be of scientific interest.

For example, the chapter by Fliederbaum *et al.* on metabolic changes records that in severely malnourished patients BMR was 30–40% below normal and was not stimulated by protein feeding, but increased by 20–50% when sugar was fed. Or again, Fajgenblat's brief report on ocular changes in starvation, or Apfelbaum-Kiwalski's report on the pathophysiology of the circulatory system in starvation, will be read with interest by all workers in the field. All will, like me, be saddened by the brevity of the reports: in reading the book I often found myself wishing that all the authors had reported their raw data, or referenced the exact methods by which they worked out complex indices (like the degree of normality of BMR) but, given the conditions in which they worked, one can only be grateful that anything at all has survived.

That which has survived will be accorded a place of honour amongst many analogous nutritional studies on the pathophysiology of protein-energy malnutrition in children that have been produced since the Second World War.

However there will remain one crucial difference between this book and many other postwar studies on the biochemistry of malnutrition. Most scientists studying malnutrition since the war have done so because they believed that the causes and cures of malnutrition should be sought at

the physiological level. The authors of this book had no such illusion. The cause of the malnutrition they describe is to them clear: it is the result of a systematic policy which, in isolating the Jews from the economic life of the nation, sentenced them to death. It was a policy which allowed a Jew only 800 kcal per day, under half of that allowed by the Germans even for people who did no work worth mentioning. It was in response to this policy that the authors undertook their study, not because they believed they would find a scientific cure for the Hunger Disease that this policy induced, but because all they could do as scientists was to create a memorial to the dead, by their contribution to scientific knowledge.

Is there in this a lesson for our times? In the 40 years since the Ghetto was destroyed, immense scientific effort has been put into studies of the nutritional and metabolic aspects of protein-energy malnutrition in children without doing anything to reduce the prevalence of the disease. Perhaps the heroic efforts of Milejowski and his colleagues should cause us now to focus more clearly on the cause of the disease and step outside a narrow scientific paradigm to seek a cure.

John Rivers is a Lecturer in the Department of Human Nutrition at the London School of Hygiene and Tropical Medicine, University of London, UK.

O polish'd perturbation

A.J. Meadows

Planets X and Pluto. By William G. Hoyt. Pp.302. (University of Arizona Press: Tucson, Arizona, 1980.) Hardback \$17.50; paperback \$9.50.

A YEAR from now we will be celebrating the two-hundredth anniversary of the discovery of the planet Uranus by William Herschel. Compared with, say, the Einstein centenary which occurred recently this might seem to be an event of limited interest. In terms of its contemporary impact, however, Herschel's discovery attracted as much public attention as the General Theory of Relativity did in this century. For the first time since prehistory, the scope of the Solar System had been enlarged. The natural reaction of astronomers was to look for other, as yet undiscovered, planets — a hunt which lasted for a century and a half.

Mr Hoyt's book is primarily concerned with the investigations that led to the last of these planetary discoveries — the detection of Pluto. But it is logical that he should introduce this search with a detailed discussion of the events surrounding the discovery of earlier planets before turning to Pluto itself. His account is readable, but highly detailed. Like his earlier book,

Lowell and Mars, the narrative is partly based on material in the Lowell archives. Like the earlier book, too, it is intended as a partial history of the Lowell Observatory, and so covers matters other than planets. But it is the search for Planet X — as the supposed planet beyond Neptune was labelled — that dominates the story.

The main outlines of the hunt for new planets is fairly familiar, at least up to the discovery of Neptune. But this earlier history — and especially the problems facing the theoretical prediction of Neptune's position in the sky — provides a fascinating parallel with the subsequent search for Planet X. Neptune was sought because the newly discovered Uranus stubbornly refused to follow its predicted path. The postulate of an outer planet, gravitationally perturbing Uranus, became an increasingly attractive possibility. Ultimately, two theoretical astronomers, Leverrier and Adams, independently predicted a position for this supposed planet. Their results were in good agreement with each other, and Neptune was, indeed, picked up close to the predicted point. So far, this was a major success story. But it rapidly became evident that some of the assumptions made by both Leverrier and Adams in determining the new planet's position were far removed from reality. How, then, had they managed to pinpoint its place so accurately? It was very quickly suggested that their result was purely a 'happy

accident', a conclusion which was equally quickly denied. The controversy continues today (it is, perhaps, a slight defect in this book that the subsequent analyses of the problem are not fully covered); but a modern consensus would give Leverrier and Adams the benefit of the doubt.

The search for Planet X possessed one immediate difference from the hunt for Neptune. Like the latter it was based on an examination of residuals, otherwise unaccounted for, in the orbits of the inner planets (in this case, Uranus and Neptune). The residuals now, however, were much smaller than those that had originally attracted attention to the existence of Neptune. It was less that these residuals forced a search for a new planet than that the desire to find a new planet motivated the investigation of the residuals.

The most detailed, though not the only, attack on the problem of Planet X was by Percival Lowell, and much of the book revolves round his activities. Lowell's final predictions appeared in 1915 — only a year before his death — but the astronomers at Lowell Observatory had already started on a photographic search for the supposed planet in 1905. After Lowell's death the search lapsed, only to be resumed at the end of the 1920s. It was then undertaken by Tombaugh, a new recruit to the observatory staff, and, early in 1930, he discovered Pluto close to the point indicated by Lowell's calculations.

This sounds like a repeat of the

successful hunt for Neptune. The similarity extends even further, for one of Lowell's contemporaries, W. H. Pickering, had predicted the existence of a planet at about the same place as that suggested by Lowell. (The parallel with Leverrier and Adams cannot be pushed too far, however, for Pickering enthusiastically predicted the existence of several planets at various distances from the Sun.) There was one major difficulty: both Lowell and Pickering supposed in their calculations that Planet X must be a reasonably massive body. Pluto, when found, proved to be fainter, and so presumably smaller, than expected. Was it really the predicted planet? The arguments started once again:

rather unproductively this time, because no mass was available for Pluto. The climax both of this dispute and of the book has actually only just occurred: for, in 1978, a Plutonian satellite was finally discovered. The consequences are remarkable — to quote Mr. Hoyt:

"The discovery of a Plutonian satellite [named Charon], of course, permitted the first direct and reasonably accurate determination of Pluto's mass since the planet's discovery forty-eight years ago . . . Pluto is thus a very, very small planet indeed, only about 20 percent as massive as the earth's moon, and with a probable diameter of only 1500 miles (2420 km), about two-thirds that of the earth's moon.

"The discovery of Charon, and the consequent determination of Pluto's very small mass, made it certain that Pluto could not be Percival Lowell's predicted Planet X, and thus the long controversy over this question ended."

The result is stated firmly; but it leads to the very odd conclusion that the positions of Pluto (certainly) and Neptune (possibly) were correctly predicted by accident. The Einstein centenary reminded us that God does not play dice. Perhaps the Uranus bicentenary should assure us that He does.

A.J. Meadows is Professor of Astronomy and History of Science at the University of Leicester, UK.

New astronomies

R.J. Tayler

A Source Book in Astronomy and Astrophysics, 1900-1975. Edited by K.R. Lang and O. Gingerich. Pp.922. (Harvard University Press: Cambridge, Massachusetts, and London, UK, 1980.) \$50.

THIS book provides an attractive invitation to review the great increase in our knowledge of the Universe over the present century. In 1900, most astronomers believed that the Milky Way system was the whole Universe and that the Sun was near its centre. The existence of interstellar matter was suspected but not established and it was not known that interstellar dust absorbed starlight and produced a seriously biased view of the Universe. Although spectroscopy had shown that stars contain the same chemical elements as the Earth, a quantitative discussion was impossible because the structure of atoms and the origin of spectral lines were not understood. A study of stellar structure had started but its further development required more knowledge of atomic physics and of nuclear structure, leading to an explanation of the source of stellar energy.

For the first two-thirds of the period under review, observational advances were restricted to optical astronomy. The Universe was seen to be an immense expanding system of galaxies and the structure of our Galaxy and its constituent stars and gas clouds was studied in great detail. Towards the end of this great period of optical astronomy, many astronomers believed that all the important components of the Universe had been discovered. This view has been transformed in the past twenty-five years by the development of new astronomies — radio, infrared, ultraviolet, X-ray and γ -ray — studying the 'invisible Universe', and by the discovery, for example, of quasars, pulsars, X-ray binaries, interstellar neutral hydrogen and molecules and the cosmic microwave radiation. Theoreticians have

used developments in physics, such as quantum theory and general relativity, to explain the properties of objects already known and to predict the existence of new types of object.

Drs Lang and Gingerich have selected about 150 key papers to trace the increase in our knowledge. In some cases the papers are printed in full and in others the less essential parts of the discussion have been paraphrased. In every instance, they have introduced their selection by some general remarks on the development of the subject; this enables them to give due credit for key work which they could not include in the anthology. I find this a fascinating collection of papers and I am impressed by

the quality of the editorial work. In reading the book, I have learnt many things which I had not previously known and others which I had forgotten. The book should certainly be widely available to both young and old students of astronomy. It is inevitable that not all astronomers will agree with the choice of key papers. In general, I did find what I expected but I was surprised that, although the work of Chandrasekhar is widely quoted, not one of his papers is included and I would have included Parker's paper on the solar wind.

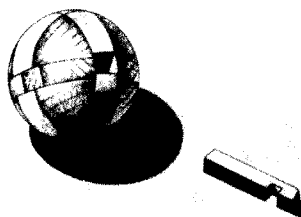
R.J. Tayler is Professor of Astronomy and Director of the Astronomy Centre at the University of Sussex, Brighton, UK.

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- MARSHALL, A.W. and OLKIN, I. Inequalities: Theory of Majorization and Its Applications. Mathematics in Science and Engineering Volume 143. Pp.xx+569. ISBN-0-12-473750-1. (New York, London, Toronto, Sydney, San Francisco: Academic Press, 1979.) \$49.50.

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- 14 May, **Measurement Standards in Respect of both Ionizing and non-Ionizing Radiation Hazards**, Teddington (British Institute of Radiology, 32 Welbeck St, London W1, UK).
- 15 May, **AGM of the British Institute of Radiology**, London (British Institute of Radiology, 32 Welbeck St, London W1, UK).
- 16 May, **Biological Effects of Doses below 2 Gray**, London (British Institute of Radiology, 32 Welbeck St, London W1, UK).
- 23 May, **Recombinant DNA Technology**, Edinburgh (Executive Secretary, The Royal Society of Edinburgh, 22 George St, Edinburgh, UK).
- 27-30 May, **Conserving our Resources — The Contribution of Chemical Technology**, Hamburg (Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).
- 28 May, **Nutrition and Killer Diseases**, London (Dr J. Rose, c/o College of Technology, Feildel St, Blackburn, Lancashire, UK).
- 28-31 May, **Water and Related Land Resource Systems**, Cleveland, Ohio, (IFAC Symposium '80, c/o AGU, 2000 Florida Ave., N.W., Washington, DC 20009).
- 2 June, **NMR Tomography: Imaging of Intact Biological Systems by NMR Spectroscopy**, Edinburgh (Executive Secretary, The Royal Society of Edinburgh, 22 George St, Edinburgh, UK).
- 2-5 June, **7th Ocean Energy Conference**, Washington DC (Gibbs & Cox Inc., 2341 Jefferson Davis Highway, Suite 1020, Century Building, Arlington Virginia 22202).
- 2-6 June, **Oil Pollution Control**, Ipswich (Miss I. McCann, Institute of Petroleum, 61 New Cavendish St, London W1, UK).
- 5 June, **Handling Product Information**, London (Aslib, 3 Belgrave Square, London SW1, UK).
- 6 June, **The Techniques of Reference Work**, London (Aslib, 3 Belgrave Square, London SW1, UK).
- 9-11 June, **How to get the Best Results from your Liquid Scintillation Counter**, Illinois (Packard Instrument 2200 Warrenville Rd, Downers Grove, Illinois 60515).
- 9-12 June, **3rd International Congress on the Menopause**, Ostend (The International Menopause Society, 8 av. Don Bosco, 1150 Brussels, Belgium).
- 10-11 June, **Technology and Challenge of the World's New Fisheries Regime**, London, (Society for Underwater Technology, London SW1, UK).
- 15-20 June, **Les Systemes de Conversion Thermodynamique de l'Energie Solaire**, Marseille (CNRS, 282 Boulevard Saint-Germain 75007 Paris, France).
- 16-17 June, **Computer-Produced Indexes**, London (Aslib, 3 Belgrave Square, London SW1, UK).
- 16-20 June, **Genetic Epistemology and Cognitive Science**, Geneva (University of Geneva, Archives Jean Piaget, 6, rue de Saussure, 1211 Geneva 4, Switzerland).
- 18 June, **AGM of the Royal Meteorological Society**, London (Royal Meteorological Society, James Glashier House, Grenville Place, Bracknell, Berkshire, UK).
- 23 Jun, **Rockstore 80**, Stockholm (Rockstore 80, c/o Stockholm Convention Bureau, Jakobs Torg 3, S-111 52 Stockholm, Sweden).
- 23-27 Jly, **The 1980 Paris-Villejuif Immunology Week (ICIG, 14 & 16 Ave Pat- Vaillant-Couturier, 94800 Villejuif, France)**.
- 23 June-1 July, **From Nuclei to Particles**, Varenna (Prof. A. Molinari, Istituto di Fisica Teorica dell'Università, Corso M. d'Azeglio 46 10125 Torino, Italy).
- 24 June, **We Need for Higher Quality Water**, London (BWETPA, 27 Crendon St, High Wycombe, Bucks, UK).
- 30 June-1 July, **Oligozoospermia**, L'Aquila (G. Frajese, Clinica Medica V-Policlinico Umberto I, 00100, Rome, Italy).
- 25 June, **The Production and Device Application of Irradiation Induced Defects in Semiconductors**, London (The Institute of Physics, 47 Belgrave Square, London SW1, UK).
- 30 June-4 July, **Electrophoretic Techniques**, Sussex (Miss L. Hart, Education Dept, Chemical Society, Burlington House, London W1, UK).
- 30 June-4 July, **International Conference on Evaluation Science and Technology—Theory and Practice**, Dubrovnik (A. Dulcic, 'Rude Boskovic' Institute, 41001 Zagreb, Croatia Yugoslavia).
- 5 July, **Regression of Atherosclerosis in Nonhuman Primates**, Florence (M.R. Malinow, Oregon Regional Primate Research Center 505 N.W. 185th Ave, Beaverton, Oregon 97005).
- 6-11 July, **Radioimmunoassay Workshop**, Guildford (Suprregional Assay Service Course Secretary, Division of Clinical Biochemistry, Dept of Biochemistry, University of Surrey, Stag Hill, Guildford, UK).
- 7-9 July, **Radio spectrum Conservation Techniques**, London (The IEE, Savoy Place, London WC2, UK).
- 9-19 July, **Topic in Ocean Physics**, Varenna (Dr A. R. Osborne, Exxon Production Research Co, PO Box 2189, Houston Texas 77011).
- 7-11 July, **An Introductory Course in Tropical Hygiene**, London (Ross Institute of Tropical Hygiene, London School of Hygiene and Tropical Medicine, Keppel St, (Gower St), London WC1, UK).
- 14-23 July, **Control of the Growth and Function of Differentiated and Cancer Cells by Intracellular Signals**, Nivelles (NATO Course IRIBHN, School of Medicine, Université Libre de Bruxelles, 2 rue Evers, B-1000 Brussels, Belgium).
- 19-25 July, **AGM of the American Malacological Union**, Louisville (Dr C. F. E. Roper, Division of Mollusks, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560).
- 21-23 July, **Soft Ionisation Methods in the Analysis of Biological Substrates**, London (Miss L. Hart, The Chemical Society, Burlington House, Piccadilly, London W1).
- 21-26 July, **4th International Congress of Immunology**, Paris (Immunology — Congress Services, 1, rue Jules Lefebvre, F-75009 Paris, France).
- 21-30 July, **The Biology of Aggression**, Toulouse, (Dr P. F. Brain, Dept of Zoology, University College of Swansea, Singleton Park, Swansea, UK).
- 2-25 July, **7th International Symposium on Olfaction and Taste, 4th Congress of the European Chemoreception Research Organisation**, Noordwijkerhout (Dr H. Van der Starre, Zoological Laboratory, University of Leiden, 63 Kaiserstraat, 2311 G Leiden, Holland).
- 21 July-2 August, **Theory of Fundamental Interactions**, Varenna (Prof. F. Paccanoni, Istituto di Fisica dell'Università, Via Marzola, 8, 35100 Padova, Italy).
- 4-7 August, **Tryptophan Metabolism: Biochemistry, Pathology and Regulation**, Kyoto (Prof. Ryo Kido, ISTRY-80 KYOTO, Wakayama Medical College, Wakayama 640, Japan).
- 1-15 August, **Muon Spin Rotation**, Vancouver (μSR2 Conference Secretariat, c/o TRIUMF, University of British Columbia, Vancouver, British Columbia, Canada V6T 2A3).
- 17-30 August, **New Developments in Membrane Research and Biological Energy Transduction**, Spetsai (Dr G. C. Papageorgiou, Dept of Biology, Nuclear Research Center "Demokritos", Aghia Paraskevi, Attiki, Greece).
- 18-22 August, **Organic Geochemistry**, New Hampshire (Dr A. M. Cruickshank, Pastore Chemical Laboratory, University of Rhode Island, Kingston, Rhode Island 02881).
- 24-29 August, **EGS Budapest '80** Geophysical Dept, Eötvös University, H-1083 Budapest, Kun Béla tér 2, Hungary).
- 27-29 August, **Water Supply and Health**, Noordwijkerhout (Symposium WSH, c/o KIVI, PO Box 30424, 2500 GK The Hague, The Netherlands).
- 27-30 August, **Chemistry, Pharmacology and Clinical Application of Nitroimidazoles**, Cesenatico (Dr M. D'Angelantonio, via Zanolini 3, 40126 Bologna, Italy).
- 27 August-6 September, **Radiosensitizers of Hypoxic Cells**, Cesenatico (Dr M. D'Angelantonio, via Zanolini 3, 40126 Bologna, Italy).
- 31 August-5 September, **Immunoassay Workshop — Advanced Course**, Guildford (Courses Secretary, Dept of Biochemistry, University of Surrey, Stag Hill, Guildford, UK).

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31 August-5 September, **Cell Biology**, Berlin (German Convention Service, Congress Organization, Joachimstaler Straße 19, D-1000 Berlin 15, FRG).

31 August-6 September, **Global Impacts of Applied Microbiology**, Lagos (Prof. O. Ogunbi, Giam VI, Dept of Microbiology and Parasitology, Lagos University, P M Bag 12003 Lagos, Nigeria).

1-5 September, **Annual Meeting of the British Association for the Advancement of Science**, Salford (Miss J.H. Dring, BAAS, 23 Saville Row, London W1, UK).

2 September, **Meat in Nutrition and Health**, Colorado Springs (T. J. McDermott, American Meat Science Association, 444 N Michigan Ave, Chicago, Illinois 60611).

2-5 September, **Inborn Errors of Metabolism in Humans**, Infrakei (Mrs S.R. Wyss, Medizinisch-chemisches Institut der Universität, Bhlstraße 28, Postfach, CH-3000 Bern 9, Switzerland).

2-5 September, **Medical Librarianship**, Belgrade (Dr L. Sablic, 1000 Bograd, Yugoslavia).

2-5 September, **Medicinal Chemistry**, Torremolinos (Secretariat: II Int Symp. on Medicinal Chemistry, 12 Oxford Rd, Cowley, Oxford, UK).

1-5 September, **5th Annual Symposium of the Uranium Institute**, London (Mrs Cherry Wilson, Conference Associates UIS, 34 Stanford Rd, London W, UK).

5-6 September, **Mammography '80**, London (The British Institute of Radiology, 32 Welbeck St, London W1, UK).

5-15 September, **Basic Principles and Methods in Membrane Bienergetics. Light Energy Transduction by Bacteriorhodopsin**, Szeged (Dr Zs. Dancshazy, Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, P O Box 521, 6701 Szeged, Hungary).

8-11 September, **Gas Discharges and Their Applications**, Edinburgh (The Institution of Electrical Engineers, Savoy Place, London WC2, UK).

8-12 September, **World Energy Conference**, Munich (Organizing Committee 11th World Energy Conference 1980, Lindemannstrasse 13, D-4000 Düsseldorf 1, FRG).

9-10 September, **Integration of Metabolism**, Nottingham (The Nutrition Society, Chandos House, 2 Queen Anne St, London W1, UK).

9-11 September, **Stress in Fish** Norwich (Dr A. D. Pickering, Freshwater Biological Association, The Ferry House, Ambleside, Cumbria, UK).

15-19 September, **The Nationwide Provision and Use of Information**, Sheffield (The Aslib/IIS/IA Joint Conference Organiser, 3 Belgrave Square, London SW1, UK).

15-19 September, **Energy Conservation & the Use of Solar & the Renewable Energies in Agriculture, Horticulture & Fishculture**,

London (Rosemary Sweeney, International Seminar Secretariat (SCU), Polytechnic of Central London, 35 Marylebone Rd, London NW1, UK).

16-19 September, **4th European Neuroscience Meeting**, Brighton (Meeting Secretariat, 142-144 Oxford Rd, Crowley, Oxford, UK).

19-23 September, **Workshop on Phytophthora Diseases of Tropical Cultivated Plants**, Kerala (Dr KKN Nambiar, Central Plantation Crops Research Institute, Kasaragod-670 124, Kerala, India).

22-23 September, **Islet-Pancreas Transplantation and Artificial Pancreas**, Athens (Prof. Dr S. Raptis, Diabetes Center and Artificial Pancreas Unit, Dept Clinical Therapeutics, Athens University, PO Box 3127, Athens 606, Greece).

22-23 September, **Human Mutation: Biological and Population Aspects**, Albany (I. H. Porter, Director, Birth Defects Institute, New York State Health Dept, Rockefeller Empire State Plaza Tower, Albany, New York 12237).

22-24 September, **Scanned Image Microscopy**, London (The Rank Prize Funds, 12 Warwick Square, London SW1, UK).

22-25 September, **Smoke Control Energy Noise Road Vehicles**, Bournemouth (National Society for Clean Air, 136 North St, Brighton, UK).

22-26 September, **History of Oceanography**, Woods Hole (J. H. Steele, 3rd International Conference on the History of Oceanography, Woods Hole Oceanographic Institute, Woods Hole, Massachusetts 02543).

25 September, **Effects of Low Temperature on Biological Membranes**, London (Dr G J Morris, Society of Low Temperature Biology, CCAP, 36 Storey's Way, Cambridge, UK).

26 September, **Food Dehydration**, Kansas (D Y C Fung, Dept of Animal Sciences and Industry, Call Hall, Manhattan, Kansas 66506).

1-4 October, **Hormones and Cell Regulation**, Bischenberg (Prof. B. Jeanenoud, Laboratoire de Recherches Médicales, Université de Genève, Ave de la Roseie, 64, 1205 Genève, Suisse).

5-10 October, **European Workshop on Drug Metabolism**, Zurich (Administrative Secretariat, PO Box 182, CH-4013 Basle, Switzerland).

6-9 October, **10th International Laser Radar Conference**, Maryland (Dr P. T. Woods, Dept of Industry, National Physical Laboratory, Teddington, Middlesex, UK).

7-10 October, **Communications and Transportation: Problems and Prospects for the 80s**, Genoa (IIC, via Pertinace, Villa Piaggio, 16125 Genoa, Italy).

7-9 October, **Polynuclear Aromatic Hydrocarbons**, Columbus (Dr A. Bjorseth, Battelle's Columbus Laboratories, 56 King Ave, Columbus, Ohio 43201).